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BACTERIA.

BY

DR. ANTOINE MAGNIN,

LICENTIATE OF NATURAL SCIENCES; CHIEF OF THE PRACTICAL LABORS IN NATURAL HISTORY TO THE FACULTY OF MEDICINE OF LYONS; LAUREATE OF THE FACULTY OF MEDICINE OF PARIS (SILVER MEDAL, 1876); GENERAL SECRETARY OF THE BOTANICAL SOCIETY OF LYONS; MEMBER OF THE BOTANICAL SOCIETY OF FRANCE; ETC.

AND

GEORGE M. STERNBERG, M.D., F.R.M.S.,

MAJOR AND SURGEON U. S. ARMY; MEMBER OF THE BIOLOGICAL SOCIETY OF WASHINGTON; LATE MEMBER OF THE HAVANA YELLOW FEVER COMMISSION OF THE NATIONAL BOARD OF HEALTH; CORRESPONDING MEMBER OF THE EPIDEMIOLOGICAL SOCIETY OF LONDON; ETC.

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PREFACE.

THE work of Dr. Magnin, which was published in Paris in 1878 and translated by the writer in 1880, gave an admirable *résumé* of our knowledge of the Bacteria at the date of its publication. But very considerable progress has been made since, especially as regards methods of manipulation, the comparative value of various chemical reagents as "germicides" and antiseptics, and the *rôle* of the Bacteria in infectious diseases. With a view to keeping the work fully up to the progress of science in this direction, the writer has added a chapter upon each of these subjects, and one upon "Bacteria in Surgical Lesions" (Parts Third, Fourth, Fifth, and Sixth). His name, therefore, appears upon the titlepage as one of the authors of the present volume. It has not been considered necessary, however, to rewrite the chapters on Morphology and Physiology (Parts First and Second). It is true that the classification of Cohn, which was very properly adopted by Professor Magnin, is only provisional, and that certain recently discovered pathogenic species are not included. But these will receive attention in Part Fifth of the present volume; and it would be premature to attempt a natural and permanent classification of these minute plants, which are now engaging the attention of numerous investigators in all parts of the civilized world. For the present we probably cannot do better than to adhere to the artificial classification, based upon morphological characters alone, which Cohn has given us.

It must be remembered, however, that SPHERO-BACTERIA — micrococci — are not always round; that there is no well-defined line of demarcation between the MICRO-BACTERIA and the DESMO-BACTERIA, between the genus BACTERIUM and the genus BACILLUS, or between the last-named genus and the LEPTOTHRIX.

The systematic naturalist, in his attempt to establish genera and species among these lowly organisms, meets with difficulties even greater than those encountered in the classification of the higher cryptogams and flowering plants. These difficulties arise from the multitude of species and minute size of the unicellular organisms under consideration; from the various phases which the same species may present at different epochs in the life-history of the plant; from the morphological identity of species having different physiological characters; and, finally, from the influence of the environment in modifying both morphological and physiological characters.

Most writers continue to speak of the Bacteria as fungi. The observations of the writer are, however, in favor of the view of Cohn, that they are more nearly related to the algae. It would be idle, however, to discuss this question, as the border-line of these two great classes of the VEGETABLE KINGDOM is not well defined; and here, as in the attempt to establish genera and species, the systematic naturalist must ever encounter the stubborn fact that NATURE is continuous, and, consequently, that all attempts at classification are artificial.

The writer ventures to hope that the *résumé* given in the present volume will be found to fairly represent the present state of science as regards the minute organisms of which it treats. No doubt the book contains much that will not bear rigid scientific criticism; and the constant additions to our knowledge which are daily being made will necessitate frequent revisions and additions, if a favorable reception by the Medical Profession, and students of Biology, makes it practicable for the writer to carry out his present intention of representing in future editions the progress which may be made in the interval. I am not prepared to say, however, that the heliotype plates which illustrate this edition will appear in subsequent editions, if they are called for. These plates add greatly to the cost of the volume, and they will perhaps be less satisfactory than lithographs or wood-cuts to those not accustomed to similar views under the microscope, and to those critics who are not familiar with the technical difficulties attending an attempt to photograph the minute organisms here represented. If the clean field and sharply-drawn outlines which it is so easy to draw upon wood or stone makes a prettier picture, and one which may be preferred by some, there can be no doubt that these views from nature, if closely studied, are more instructive than drawings, notwithstanding the inevitable defects arising in some instances from the presence in the field of view of extraneous objects, and from the impossibility of having every part of the field in the best possible focus at the same time in these photo-micrographs, which are made with objectives of high power having an extremely limited focal range.

G. M. S.

Fort Mason, San Francisco,
August 15, 1883.

INTRODUCTION.

PART FIRST.

MORPHOLOGY OF THE BACTERIA.

PART SECOND.

PHYSIOLOGY OF THE BACTERIA.

BY

DR. ANTOINE MAGNIN.

TRANSLATED FROM THE FRENCH

BY DR. GEORGE M. STERNBERG.

PREFACE BY TRANSLATOR.

HAVING found the admirable *resumé* of our knowledge of the bacteria, by Dr. Magnin, of great assistance to me, in pursuing the investigations in which I have been engaged during the past year under the auspices of the National Board of Health, it has seemed to me that a translation of the work into English and its publication in this country would be productive of good in more ways than one, and of the advancement of science. To the naturalist, it cannot fail to be of value, as the most approved classification, that of Cohn, is given, with a full description of species. To give additional value to this portion of the work, figures of many of the best-known forms, drawn from various foreign sources, and reproductions of some of my own photo-micrographs (by permission of the National Board of Health), have been introduced.

If we are to judge from the scanty literature of the subject in this country, the amount of interest which has been aroused by the revelation of a new world of micro-organisms, and by the momentous questions which have been raised in connection with them, is far below that awakened in Germany, France, and England. This is not, however, really the case; for, while we have but few active workers in the difficult fields of inquiry which have proved so attractive, especially for the German and the French *savants*, there is nevertheless a wide-spread interest in these investigations, and a desire to know their results. But, just here, we are met with a difficulty which has no doubt discouraged many, and perhaps caused some to drop the whole subject in disgust. The results have been so contradictory, and so many would-be *savants* have uttered opinions entirely opposed the one to the other, that we find it impossible to arrive at any definite opinion, not knowing whom to believe. This being the condition of affairs, it seems to me that it is necessary for us to commence investigating for ourselves, — first making ourselves familiar with what has been done abroad, and then avoiding, if possible, the quicksands into which unfortunate science has too often been dragged by her votaries. One great trouble which we have experienced in this country is in judging of the comparative value of the observations of different men who are equally unknown to us. A very plausible article may be written by a very careless observer; or a very cautious observer may fail to give confidence in his results, because of a certain degree of confusion in his language. When experiments are well devised, carefully executed, and described with precision, as is done by such men as Pasteur and Tyndall, we cannot fail to attach great weight to the conclusions reached. And when so accomplished a microscopist as Cohn or Koch asserts that he has seen such and such a thing, or has made such and such measurements, we cannot doubt the reliability of the observation. But sometimes we are deceived by giving credence to a man who has achieved reputation in one line of study, but of whose skill and training in the use of the microscope we have no means of judging. Such a man may be a great surgeon, or a great clinician, or a great chemist, and yet be a mere tyro with the microscope. When, then, we see it announced that Dr. So-and-so failed to discover any *micrococci* in pus, in blood, or what not, taken from a certain source, we are justified in asking, — first, what power did the learned doctor use? second, is he capable of distinguishing micrococci in fluids which contain them beyond question? Or, if he does discover them, we may ask if he is accustomed to making a differential diagnosis between micrococci and inorganic granular material, or unorganized granules of organic origin. This is a decision which the most accomplished microscopist is sometimes unable to make, except by the aid of chemical tests and culture experiments.

To avoid this want of confidence in results, which has naturally grown out of carelessly made observations and contradictory statements, it is desirable that full and minute details should be given of all observations and experiments made, and, whenever possible, that photomicrographs should be made of all micro-organisms described, or of a thin stratum of a liquid asserted not to contain any; as, when a sufficiently high power is used, this settles the question of their presence or absence, beyond dispute, and enables other students to make comparisons and measurements which cannot fail to promote the interests of true science.

The National Board of Health of the United States has the credit of first adopting this method of recording the results of scientific investigation, in this direction, as a constant and unimpeachable record of what has been seen by the investigator. The commission sent to Havana last summer for the investigation of yellow fever, was instructed to pursue this method, and was accompanied by a photographer and supplied with all the necessary appliances for carrying these instructions into effect.

The superficial reader may find much to criticise in the work of Dr. Magnin, but I am convinced that those who read it carefully cannot fail to be pleased with the truly scientific spirit in which it is written; the fairness with which conflicting opinions are stated; the caution manifested as to the drawing of definite conclusions where questions are still under discussion; and, above all, the extent of his literary researches and the systematic way in which he has arranged the results.

For the naturalist, for the physician, or for the non-professional man of general culture, who desires to have accessible in a condensed form the most important results achieved in this line of inquiry up to the present day, this volume cannot fail to be of value; while for the student and the investigator in search of fuller information, the summary given of the labors of numerous individuals, together with the copious bibliography, which I have brought up to date, will doubtless be of service. Believing this to be true, it has been a pleasure for me to devote a portion of my summer vacation to the translation of this little volume.

G. M. S.

Salem, Mass., August 1, 1880.

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THE BACTERIA.

INTRODUCTION.

"Corruptio unius est generatio alterius."
LUCRETIUS, *De Rerum Natura.*

OF all the studies which have for their object the inferior organisms, those which relate to the bacteria offer, without contradiction, the greatest interest, as they touch the most diverse problems, which, it is true, are the most difficult and the least known in biology. The history of these minute organisms is, in truth, related to that of spontaneous generation, to that of the fermentations, to the pathogeny and therapeutics of a great number of virulent and contagious affections, and, in a more general manner, to all the unknown which, notwithstanding the efforts of modern science, still surrounds the origin of life and its preservation.

If the relation of these inferior organisms to the origin of living beings is yet obscure, their *rôle* in the preservation of life is better known. It is known that organic matter, once produced and become solid, so to speak, cannot again enter into the general current until it has undergone new transformations, metamorphoses produced, according to some *savants*, favored, according to others, but, without contradiction, accompanied by the development of bacteria;[1] and, without wishing to attribute to these organisms a finality which is repugnant to our monistic conception of the universe, it may be said that it is thanks to them that the continuation of life is possible on the surface of the globe.

But, if these studies are full of interest, their field is so vast that we cannot flatter ourselves that we have passed over the whole of it with equal care. The little time that has been accorded us for the composition of this thesis will be our excuse for the inevitable imperfections which will doubtless be found in our work.

---

[1] *The bacteria:* such is the subject which has been imposed upon us; but it is certainly useless to give the reasons which have caused us to study not only the bacteria properly so called, taking the word in its most restricted sense, but all the organisms which are comprised under the names of bacteria, vibrios, schizomycétes, schizophytes, etc.

HISTORICAL.

THE bacteria are the lowest organisms, situated upon the limit of the two kingdoms, animal and vegetable, and are thus defined by the botanists who have most recently occupied themselves with them: —

"Cells deprived of chlorophyll, of globular, oblong, or cylindrical form, sometimes sinuous and twisted, reproducing themselves exclusively by transverse division,[1] living isolated or in cellular families, and having affinities which approach them to the algæ and especially to the oscillatoriæ."

But, before arriving at this degree of relative precision, the history of the bacteria has passed through the most diverse vicissitudes. At one time considered as animals, at another taken for vegetables, transported from the algæ to the fungi, one author has even gone so far as to refuse to them the nature of living beings.[2] This diversity of opinions is due to the minuteness of their dimensions and the difficulties with which their observation is surrounded.

---

[1] Reproduction by spores has been proved to occur in *Bacillus subtilis*, and it seems altogether probable that other species are reproduced in the same way. — G. M. S.

[2] Polotebnow.

Although an historical statement of the progress of our knowledge of these minute organisms has been given in several publications, we think it best to make here a new historical summary, which will be completed by an indication of the principal papers relating to them which have been published recently.

The first observer who perceived bacteria was Leeuwenhoeck. As early as 1675, while examining by chance with his magnifying glasses a drop of putrid water, the father of microscopy remarked with profound astonishment that it contained a multitude of little globules, which moved with agility. The following year he recognized the presence of bacteria in fæces and in tartar from the teeth; and, if he has not named them, it is easy to assure one's self by the description which he has given of their form and of their movements, and by the figures which accompany these descriptions,[1] that the organisms observed by him are truly *Bacteria*, *Vibrios*, and perhaps even *Leptothrix*.

In 1773 O. F. Müller endeavored to classify these organisms. He made of them a group of infusoria, under the name of *Infusoria crassiuscula*, and established two genera, — the *g. Monas* and *Vibrio;* the first characterized as follows: "*vermis inconspicuus*, *simplicissimus*, *pellucidus*, *punctiformis*," comprising the following species: *Monas termo*, *atomus*, *punctum*, *ocellus*, *lens*, *mica*,

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[1] Leeuwenhoeck. *Opera omnia*, Lugd. Batav., 1722, 11, p. 40, fig. A to G.

tranquilla, lamellula, pulvisculus, uva, which it is impossible to identify with the species at present recognized. The genus *Vibrio* "*vermis inconspicuus, simplicissimus, teres, elongatus,*" enclosing under thirty-five specific names, with the true bacteria, some organisms belonging to other classes of the animal and vegetable kingdoms.

In the classification of the infusoria given by Bory de Saint-Vincent in the "Encyclopédie Méthodique" (1824) and afterwards in the "Dictionaire Classique d'Histoire Naturelle" (1830) the bacteria are distributed in two different families of the microscopic gymnodæ, the monadaires and the vibrionides. Besides the *monads,* properly so called, of which the *Monas termo* has been preserved by the greater part of the bacterologists, the *monadaires* include some veritable infusoria, which have no relation with the monads. It was the same with the vibrionides, of which the genera *Vibrio* and *Mellanella* included some beings very different in their organization. Indeed, beside some veritable *vibrios, bacteria,* and *spirilla,* constituting the genus *Mellanella,* Bory placed some nematoid worms, such as the *Anguillula* of vinegar.

With Ehrenberg (1838) and Dujardin (1841) the family of the vibrioniens was established upon characters more homogeneous, and their species upon distinctions truly scientific. But these two observers, followed in this by M. Davaine, deny completely the affinities of the elongated bacteria

(*Bacterium*, *Vibrio*, etc.) with the punctiform bacteria (*Monas*); and it is necessary to come to the time of MM. Hallier, Hoffmann, Cohn, and the greater number of recent botanists, in order to see these two forms brought together anew. In fact, Ehrenberg defines his vibrioniens, which he arranges between the volvocineæ and the closteria "animals, filiform, distinctly or apparently polygastric, no mucous membrane, naked, without external organs, with the body (like monads) uniform and united in chains or filiform series, as a result of incomplete division." He included in this class all filiform bodies gifted with proper movement and formed of articles, dividing them into four genera: —

1. *Bacterium:* filaments linear and inflexible; three species.
2. *Vibrio:* filaments linear, snakelike, flexible; nine species.
3. *Spirillum:* filaments spiral, inflexible; three species.
4. *Spirochæte:* filaments spiral, flexible; one species.

A fifth genus, including but one species, the *Spirodiscus fulvus*, with filaments in a helix, inflexible, disposed in contiguous layers, has not been seen since Ehrenberg. Let us add that Ehrenberg often attributed to them a complex structure, stomachs more or less numerous, a proboscis, cilia serving as organs of locomotion, — all characters that more recent observers have failed to find. Nevertheless, we must make an exception in favor of the cilia, of which the existence has been recently verified in the case of several of the bacteria by divers botanists, among others by MM. Cohn and Eug. Warming.

Dujardin (1841), in his "Histoire Naturelle des Zoophytes," preserved the family of the vibrioniens of Ehrenberg among the infusoria, characterizing them as follows: "filiform animals, extremely slender, without appreciable organization, without visible locomotive organs." He made but few modifications, of which the principal consisted in uniting *Spirochæta* with *Spirillum*, Dujardin. Rejecting the character that Ehrenberg drew from the rigidity of the spirilla, the *Spirochæta plicatilis*, Ehrb. became the *Spirillum plicatile*, Duj.; but, as will be seen later, this change has not been maintained. Dujardin, then, classed the bacteria in:

1. *Bacterium:* filaments rigid, with a vacillating movement.
2. *Vibrio:* filaments flexible, with an undulatory movement.
3. *Spirillum:* filaments spiral, movement rotatory.

Until this time the bacteria had been considered as animals placed at the foot of the series. Subsequently the tendency to place them in the vegetable kingdom became more and more pronounced.

Already, since 1853, M. Ch. Robin had pointed out the relationship of the bacteria and of the vibrios with *Leptothrix*. This opinion, which was not favorably received by the authors who adopted nearly all of the generic groups of Ehrenberg and Dujardin, is to-day accepted by many botanists, above all since the labors of Cohn. (See below: *classification*.) At all events, it is to M. Davaine (1859) that we are indebted for clearly pointing out that the vibrioniens are vegetables, nearly allied to the algæ, and especially to the confervæ.

This same author, having observed some motionless bacteria, thought it necessary to give this character great consideration, and to establish a fourth group, the genus *Bacteridium*, which he added to the three others admitted by Dujardin; but in this creation he was less happy than in his placing the vibrioniens among the vegetables; for we shall see further on that this character of mobility or of immobility is not absolute, and that it depends upon the age of the bacterium or upon certain conditions relating to the medium in which it is placed.

The most recent complete exposition of the classification and of the ideas of M. Davaine is found in the "Dictionnaire Encyclop. des Sciences Médicales," art. Bactéries (1868). It may be summed up as follows:—

Filaments straight or bent, but not in a spiral	Moving spontaneously	Rigid	BACTERIUM.
		Flexible	VIBRIO.
	Motionless		BACTERIDIUM.
Filaments spiral			SPIRILLUM.

The genus *Bacterium* comprises six species,—*B. termo*, *catenula*, *punctum*, *triloculare*, or *articulatum*, already described by Ehrenberg and Dujardin, and *B. putredinis* and *capitatum*, new species of M. Davaine, established, the first for a bacterium producing rot in plants, the second for a species, swollen at the extremity, observed in some macerations.

The genus *Vibrio* includes twelve species,—*V. lineola*, *tremulans*, *rugula*, *prolifer*, *serpens*, *bacillus*, *synxanthus*, and *syncyanus* of previous authors and the *V. lactic*, *butyric*, and *tartaric right*, discovered by M. Pasteur in these different fermentations.

In the genus *Bacteridium*, M. Davaine places five new species,—the "*Bactéridies charbonneuse*, *intestinale*, *du levain*, *glaireuse*, *et des infusions*." He includes also the ferment which, according to M. Pasteur, occasions the "sickness of turned wine."

Finally, the genus *Spirillum* includes the species *S. undula*, *tenue*, *volutans* of Ehrenberg, *S. rufum* and *leucomænum* of Perty, and *S. plicatile*, Duj.

From this moment the history of the bacteria enters upon a new phase. The labors of M. Pasteur upon the inferior organisms and their *rôle* in fermentation, the researches of MM. Davaine and Hallier upon the bacterium of charbon, and the micrococci of contagious maladies, call the attention of chemists and of pathologists to these organisms and especially to the bacteria. Their origin, their evolution, the physiological peculiarities of their nutrition and reproduction, are the object of numerous labors, and give rise to passionate discussions relating to the subject of spontaneous generation, polymorphism of fungi, theories of fermentation, and the pathology of virulent and infectious maladies. For this reason an exposition of these researches, often contradictory, is extremely difficult. We will make it succinctly, insisting especially upon the labors relating to the classification of the bacteria, and reserving to ourselves the privilege of returning to the history of several points, when we approach their study in the special chapters of this thesis.

The first important memoir published after that of M. Davaine upon the bacteria is that of M. Hoffmann, in 1869. He demonstrates: *First*, that the bacteria are plants, having a very distinct cellular organization; *second*, that they can only be classified in accordance with their form and size, at first into monads and linear bacteria, and the latter into microbacteria, mesobacteria, and megabacteria; (M. Hoffmann includes with the linear bacteria, *Vibrio*, *Bacterium*, and *Leptothrix*, which are bacteria united in a chaplet;) *third*, that mobility or immobility is not a specific character, but may present itself in the same species under the influence of changes of temperature, of density of medium, etc. M. Hoffmann studies also the origin of the bacteria, and rejects the hypothesis of a spontaneous generation. As to their *rôle* in the phenomena of the decomposition of organic bodies and in fermentations, M. Hoffmann confesses "that, with the exception of yeast and of the acetic and butyric ferments, all the rest is still enveloped in obscurity."

M. Cohn is the naturalist who, in our days, has occupied himself the most with the bacteria. In 1853, he published his first researches upon this subject. The genera *Zooglœa*, which he established at this time for the bacteria arranged in gelatinous masses, diffused or more or less crowded together, was not a happy creation. It was adopted at first by M. Rabenhorst who, in his work on the fresh-water algæ of Europe, places them after the palmellaceæ, while he classes the other bacteria, *Vibrio* and *Spirillum*, in the family of the oscillatoriæ. The *Zooglœa* are later abandoned by their author as a generic group, and are preserved only as the name of one of the diverse transitory stages through which the bacteria pass in the course of their evolution (*Zooglœa*, *Leptothrix*, *Torula*).

Twenty years later the same *savant* commenced the publication of a series of "Memoirs" upon these organisms (in his "Beiträge zur Biologie der Pflanzen"). In the first paper the author gives an exposition of his researches upon the organization, development, and classification of the bacteria, and upon their action as ferments.

M. Cohn considers them as a well-defined group, — the schizospores, belonging to the algæ, at the commencement of the series of the phycochromaceæ, with several families with which the different genera of bacteria have many affinities. He recognized, however, that the absence of chlorophyll approaches them, at least from a functional point of view, to the fungi. Upon this point we may say that for other botanists this character is decisive, and the bacteria are classed as fungi.

M. Nägeli, who takes this view, describes them under the name of *Schizomycetes*. Cohn divides the bacteria into four tribes, comprising six genera: —

1. The *Sphærobacteria* or globular B.
2. The *Microbacteria* or rod B.
3. The *Desmobacteria* or filamentous B.
4. The *Spirobacteria* or Spiral B.

We will return to this classification.

In 1874, M. Th. Billroth, in his researches upon the *Coccobacteria septica*, expressed opinions entirely different from those of Cohn. According to Billroth, the bacteria differ considerably in form according to the medium in which they are placed and divers circumstances. He claims that they constitute but a single species, the *Coccobacteria septica*. This vegetable organism can present itself under the form of globular articles (*coccos*) or under that of rods (*bactérie*). These two forms may reproduce themselves by becoming elongated and dividing transversely, or may pass the one into the other. Billroth claims to have found both forms united in a single filament, a fact which in his opinion demonstrates conclusively their relationship. Each of these two forms can also present variations of size, in accordance with which he establishes the following divisions: —

Micrococcos	Microbacteria.
Mesococcos	Mesobacteria.
Megacoccos	Megabacteria.

And varieties of association which give rise to the following names: —

Monococcos	Monobacteria.
Diplococcos	Diplobacteria.
Streptococcos	Streptobacteria.
Gliacoccos	Gliabacteria.
Petalococcos	Petalobacteria.
Ascoccos.	

The following year (1875), Cohn, in the second part of his "Researches" upon the bacteria, criticised the opinions expressed by Billroth in the preceding memoir. Cohn believes that we should regard as distinct genera and species all the bacteria having a particular form and acting differently as ferments, so long as the proof of their identity has not been demonstrated in an evident manner. Coming back also to the affinities and classification of these organisms, he insists anew upon their near relationship to the Phycochromaceæ; and, no longer distinguishing the bacteria as a special family, he distributes his different genera in a group, which he calls *Schizophytes*, which includes the greater part of the

Chrococceæ and of the *Oscillariæ.* We will return to this subject when we speak of the classification of the bacteria.

In 1876, appeared in the same number of Cohn's "Beiträge" two important papers. The first, by Cohn, treats of the influence of temperature upon the bacteria, of their origin, of the formation of spores in the *Bacillus* of hay infusion, and of charbon. The second, by Koch, gives the result of his researches upon the bacteria of charbon, the *Bacillus anthracis.*

Koch has been able by skilful cultivation to follow the complete development of this *Bacillus*, and to witness the formation of spores, of which the vitality is very great, and which are the principal agents of the transmission of this terrible malady.

I must still indicate, in addition to these special works, a quantity of notes and of memoirs scattered through the reviews and periodical publications.

The list will be found in the bibliography appended to this work. I must also cite the recent work of M. Nägeli upon "The Inferior Fungi and their *Rôle* in Infectious Maladies." The learned professor of Munich has studied the diverse fungi which produce decompositions. He divides them into three groups, — the *Mucorini*, the *Saccharomycetes*, and the *Schizomycetes*, which correspond to the bacteria. According to Nägeli, the bacteria are fungi which produce putrefaction.

In presence of these opinions, so diverse, as to the nature of the bacteria and their classification, we will finish by saying with Cohn: —

"So long as the makers of microscopes do not place at our disposal much higher powers, and, as far as possible, without immersion, we will find ourselves, in the domain of the bacteria, in the situation of a traveller who wanders in an unknown country at the hour of twilight, at the moment when the light of day no longer suffices to enable him clearly to distinguish objects, and when he is conscious that, notwithstanding all his precautions, he is liable to lose his way."

PART FIRST.

MORPHOLOGY OF THE BACTERIA.

CHAPTER I.

ORGANIZATION OF THE BACTERIA.

When bacteria develop in a liquid in a sufficient quantity, they become visible to the naked eye. They appear either as a slight cloud, or gathered in little masses in the liquid, or forming a pellicle upon its surface, or as a deposit upon the walls of the vessel and upon the objects contained in the liquid. However, we must hasten to say with M. Cohn, that the fact of the absence of all turbidity in a liquid does not exclude the possibility of the presence of bacteria. In liquids more dense than water (serum, lymph, etc.), when the refractive power of these corpuscles is the same as that of the liquid, their presence may not be revealed to the naked eye. We will add that sometimes their color serves to indicate their presence in a liquid, although this color is often very feeble, and can only be perceived when a considerable thickness of the liquid is examined. If we examine these clouds, these accumulations, these deposits, with the microscope, we see that they are formed of a myriad of little bodies isolated or grouped, globular or linear, gifted or not with motion, sometimes colored. These variations constitute so many characters which require to be studied with some detail.

§ 1. BACTERIA IN GENERAL.

Form. — The bacteria, as understood to-day by most botanists, when considered in their separate state, are of two principal forms, — globular bodies, or *monads,* and bodies more or less filiform, or *bacteria* properly so called.

The globular bacteria comprise organisms rounded, ovoid, sometimes elongating themselves into a tube (Warming). The *Monas crepusculum* of Ehrenberg may be taken as a type. This form includes also the *Micrococcus* of Hallier, the *Microsporon* of Klebs, the round forms of the *Amylobacter* of M. Trécul, and perhaps the *Microzyma* of M. Béchamp. We will see farther on that these are very probably phases of development of the spores of bacteria, properly so called.

The bacteria, not globular, present a greater diversity of form; they may be straight, undulating, or twisted in a spiral.

The rectilinear bacteria are usually exactly cylindrical throughout their whole extent; and in this case they form very short cylinders, as in the *Bacterium,* Cohn, or cylinders of which the length is several times as great as the thickness, as in the *Bactéridies* (*Bacillus ulna* Cohn); others are swollen in the middle, with their extremities rounded, such as certain forms of *Vibrio serpens* (Warming); others again are fusiform, swollen in the middle and attenuated at the extremities,—*Bacterium fusiforme* (Warming); rectilinear bacteria swollen at the two extremities are met during the life of certain species, *B. lineola* and *B. termo,* for example, above all when they are transported to a more favorable medium: this modification usually precedes segmentation; finally, one meets sometimes bacteria swollen at one extremity only; the swollen part presents often a clear point and sometimes an evident spore: we shall see later the signification of this peculiarity. With these claviform bacteria we may include the *Bacterium capitatum* Dav., the *Helobacteria* of Billroth, and certain *Amylobacter,* with heads of the *Ficus carica,* etc. (Ch. Robin).

The undulating bacteria constitute the *Vibrios* properly so called (*V. rugula, serpens,* etc.).

The spiral bacteria of which the turns are more or less elongated are named *Spirillum, Spirochæta,* etc.

Dimensions.—The dimensions of the bacteria oscillate between the most variable limits, but in a general way it may be said that they are the smallest of all microscopic beings. Some of them are situated at the extreme limit of our highest magnifying powers; and their proportions, as to length and thickness, are comprised within the limits of errors of observation.

The globular bacteria are the smallest, and the dimensions of some species are so minute that they cannot be measured directly.

The largest are the *Spirillum*, which attain a length of $\frac{2}{10}$ of a millimetre. Between these two extremes, there are all intermediary sizes possible. The dimensions of some of the bacteria are given below:—

Monas vinosa, 0.5 to 1 μ, in diameter; length 3 to 4 μ.

Bacterium termo, breadth 0.6 to 0.8 μ; length 2 to 3 μ.

Vibrio lineola, breadth 0.5 to 1 μ; length 3 to 8 μ.

Bacillus ulna, „ 0.7 to 1 μ; „ 5 to 8 μ.

B. anthracis, „ 1 to 2 μ; „ 10 to 50 μ.

Spirillum volutans, „ 7 μ; „ 10 to 40 μ.

Several authors, considering exclusively this character of dimensions, have divided the monera and the bacteria according to their size. Thus Hoffmann recognizes in addition to the monera, only the microbacteria, the mesobacteria, and the macrobacteria. In the same way Billroth classifies the monads according to their dimensions into *micro, meso, mega coccos*, and the bacteria into *micro, meso, mega bacteria.* Finally, Klebs separates the *Micrococcos* from the *Microsporines*, which do not differ from them except by their smaller dimensions, both forms being able to pass to the state of *bacteria* (rods).

Color. — The phenomena relating to the color of bacteria have only recently been pointed out. "But little attention has been given to the color of the bacteria, regarded generally as colorless," said M. de Seynes in 1874; and recently M. de Lanessan, "The bacteria are ordinarily quite colorless." However, M. Cohn had already insisted upon the globular bacteria *chromogènes*, or of pigmentary fermentation, and upon the colors produced by different monads, which have long since been studied by microscopists.

Upon this subject, let us observe that the bacteria which are colored belong to two very different groups. First, colored organisms always known as such, but which were not formerly included with the bacteria, as the different monads, which have become the *Micrococcus prodigiosus*, *cyaneus*, *aurantiacus*, Cohn, etc.; the second group includes the bacteria properly so called, which absorb the coloring matter of vegetables upon which they are fixed as parasites, or of the media in which they live. This is the case with the bacteria observed by M. de Seynes upon the *Penicillium glaucum*, and perhaps with the *Vibrio synxanthus* and *syncyanus*, Ehrenb., which give to milk a yellow or blue color according to the species. We will return to this subject when we speak of the nutrition of the bacteria.

As to the purple-colored monads, they have been especially studied as early as 1838 by Dunal, then by Morren and Ehrenberg, and in our own day by Ray-Lankester, Cohn, Klein, and finally by Warming and Giard. They are found in various media — in sea-water, in hot sulphur springs, in fresh water containing animal or vegetable matter in a state of putrefaction. They appear sometimes upon bread, meats, and in general upon cooked food placed in a humid atmosphere. The different colors which they present are red, yellow, orange, and blue. It is probably to analogous organisms that we must attribute the blue color presented by pus under certain circumstances, the green and blue color studied by M. Chalvet, and the orange-yellow, bright red, and blue colors observed by C. Eberth in perspiration.

In Norway, red bacteria appear in summer in such masses that the borders of the sea are sometimes colored of an intense red (Warming).

Movement. — The bacteria are met in two different states. They are active or motionless; but it is now well settled for the greater number that the same species may present itself sometimes in a state of repose, sometimes in a state of movement.

The movements of the bacteria are of two kinds, — a movement of the corpuscle upon itself and a movement of translation. The first is sometimes nothing more than a molecular or *brownien* movement, which occurs in the smallest forms. But at other times it is more extended, and consists in a movement of rotation round the axis, or a bending of the body. This flexibility is, above all, observed in the long forms, the *Bacillus*, the *Vibrions*, etc. As to the movement of translation, it is very variable; at one time slow, at another rapid, it is in relation with the length and form of the bacterium. M. Cohn has well described all the modifications of movement in the following lines: —

"Almost all the bacteria possess two different modes of life, characterized by repose and by movement.

"In certain conditions, they are excessively mobile; and when they swarm in a drop of water, they present an attractive spectacle, similar to that of a swarm of gnats, or an ant-hill. The bacteria advance, swimming, then retreat without turning about, or even describe circular lines. At one time they advance with the rapidity of an arrow, at another, they turn upon themselves like a top; sometimes they remain motionless for a long time, and then dart off like a flash. The long rod-bacteria twist their bodies in swimming, sometimes slowly, sometimes with address and agility, as if they tried to force for themselves a passage through obstacles. It is thus that the fish seeks its way through aquatic plants. They remain sometimes quiet, as if to repose an instant: suddenly the little rod commences to oscillate, and then to swim briskly backwards, to again throw itself forward some instants after. All of these movements are accompanied by a second movement analogous to that of a screw which moves in a nut. When the vibrios in the shape of a gimlet turn rapidly round their axis, they produce a singular illusion: one would believe that they twisted like an eel, although they are extremely rigid."

The causes of these movements have been sought, at first, in the supposed animal nature of the bacteria, and the movements assimilated, consequently, to voluntary movements; but this opinion can no longer be sustained, as similar movements are to be seen in a great number of vegetable organisms, such as the diatoms, the oscillatoriæ, the spores of algæ and some fungi, etc. They have also been attributed to the existence of locomotor appendices (Ehrenberg); but, although the cilia, denied at first by most microscopists, have been seen since in nearly all the bacteria, the botanists who have best studied them, M. Warming, for example, recognize that it is scarcely probable that these organs are the cause of their movements, for "one meets some examples in which the body remains motionless while the cilia are in violent agitation, and others in which the body moves while the cilia remain inert, or dragging behind."

The movements appear to depend rather upon the nutrition, or respiration, and especially upon the presence of oxygen (Cohn); indeed when this gas is wanting the bacteria become motionless. Immobility may also be produced by want of nutriment, poisoning by different toxic substances, (chloroform, iodine, etc.), dessication, etc.

The attempt has been made to use the characters derived from the existence or absence of motion, and the form of the bacteria, in order to classify them; but what has just been said shows clearly that these transitory phenomena cannot be taken for generic or specific characters.

Structure. — It was for a long time believed that the bacteria were constituted of amorphous masses of protoplasm, or of solid rods. The researches of Hoffmann have shown that they have a true cellular structure. We shall describe, then, successively, their membrane, the contents, and the cilia, which may be considered as belonging to the protoplasm.

Cell-membrane. — The extreme minuteness of the bacteria usually prevents a direct demonstration of the cell-membrane, and the existence of this envelope has not, heretofore, been clearly demonstrated except by indirect proofs; chemical reactions, for example.

Thus Hoffmann verifies the existence of a cellular envelope when "the contents, which is a transparent plasma, are partly coagulated, as sometimes happens, or disappear, and are then replaced by air which shows precisely the form of the normal bacterian cell." Warming, also, has not been able to see the membrane, "which only appears distinctly when a vacuole has formed just against the periphery."

On the other hand, the action of chemical agents upon bacteria proves that they have an envelope of cellulose, which is colored by tincture of iodine;

is not destroyed by caustic potash, ammonia, or even acids; and resists putrefaction for an exceedingly long time. In this respect, it resembles the membrane of cellulose of vegetable cells (Cohn).

We should add that Cohn claims to have succeeded with high powers in seeing directly the cell-membrane. On the other hand, Warming has never succeeded in so doing. The last observer remarks also that the resistance of bacteria to acids, to alkalis, etc., does not seem to prove the existence of a membrane, "inasmuch as this may be the result of a particular condition of the plasma, which in all the bacteria is of a more consistent nature than in other plants."

Finally, the membrane may be, in certain bacteria, tender, flexible and susceptible of movements of torsion. In others, it is rigid and incapable of bending. Cohn thinks also that it may swell and dissolve into mucilage, a fact which would explain the origin of this substance in the *Zooglœa.*

Protoplasm. — The contents of the cell is a nitrogenous substance, generally colorless, more highly refractive than water.

In the smallest species, this protoplasm appears homogeneous; but in the bacteria of medium size, and above all in the large species, the contents of the cell encloses portions more highly refractive, vacuoles, special granules, and sometimes diverse coloring matters.

Cohn has first pointed out the movements of the protoplasm, in which currents occur, above all in the central portion, the peripheral portion remaining homogeneous and motionless. The vacuoles are also found in the central portion; Warming, however, who has observed them in *Monas Okenii*, *Vibrio rugula*, *V. serpens* and *Spirillum undula* var. littoreum, has sometimes seen them in the middle of the plasma, at another near the exterior wall.

The granules which are seen in the protoplasm were considered by Ehrenberg as stomachal vesicles or ovules. They are of two sorts; the one, highly refractive and not bordered by a dark circle, are considered by Warming as nothing more than mere compact masses of protoplasm; the second, also highly refractive, but surrounded by a dark circle, resemble drops of oil, and have been taken for fat granules; but the recent researches of Cramer, Cohn, and Warming have proved that some of them, at least, are formed of crystalline sulphur. They are not soluble either in hydrochloric acid or in water, but they are dissolved in absolute alcohol, in hot caustic potash and sulphite of soda, in nitric acid and chlorate of potash at ordinary temperatures, and in bisulphide of carbon, when the membrane, which is permeable with difficulty, has been previously destroyed by sulphuric acid. Although their small dimensions and great refractive power prevent them from being distinguished with certainty as crystals of sulphur, as they are doubly refractive to polarized light their crystalline nature cannot be doubted.

These globules of sulphur have been observed in *Monas Okenii*, *Bacterium sulphuratum*, *Ophidomonas*, and the different species of *Beggiatoa*, both in fresh water, in putrid sea-water, and in thermal sulphur waters. It will be seen when we speak of the physiology of these organisms what their *rôle* is in the elimination of sulphur and the formation of sulphuretted hydrogen.

We have said, in speaking of the colored bacteria, that some borrow their color from the surrounding medium, and that others, on the contrary, have a color of their own. The protoplasm of the latter contains a granular coloring matter, which is ordinarily yellow, blue, or red. The red coloring matter is most common, and this has been best studied, and appears to be the best known.

One of these colors which gives a pink tint (peach color) to *Bacterium rubescens*, Ray-Lank. (*Clathrocystis roseopersicina*, Cohn); *Monas vinosa*, Ehrb., *M. Okenii*, Cohn; *M. gracilis*, Warming; *Rhabdomonas rosea*, Cohn; *M. Warmingii*, Cohn; *Ophidomonas sanguinea*, Ehrb.; *Merismopedia littoralis*, Rabenh.; etc., has been studied by Ray-Lankaster, who has given to it the name of bacterio-purpurine. It is insoluble in water, soluble in alcohol, ether, carbolic acid, glycerine, and fatty oils,—characteristics which make it resemble chlorophyll. It has also a characteristic spectrum.

Other red coloring matters which appear to be different have been found in *Monas prodigiosa*, Ehrb.; *Bacillus ruber*, Cohn; and *Micrococcus fulvus*, Cohn. These should not be confounded with the purple coloring matter of other algæ, as that of the *Porphyridium cruentum*, which comes from a mixture of chlorophyll and of phycoerythrine. The bacteria never contain chlorophyll.

In this connection, it is interesting to recall the protoplasmic constitution of the *Amylobacter* of Trécul. These organisms are, according to Van Tieghem, bacteria, to which he has given the name of *Bacillus Amylobacter*, and which does not differ from *B. subtilis*, except by a specific character, extremely transitory, — the presence of amorphous starch, formed and stored in reserve during the period of growth, to be again used later, and consumed during the process of reproduction.

Cilia.—These appendages which were described by Ehrenberg in the *Bacterium trilocular* have not been seen since. To-day, recent researches permit us to say that cilia exist without doubt in all the true bacteria, — *Bacillus*, *Bacterium*, *Spirillum*. They have been perceived in a great number of forms, — *Spirillum volutans*, *Sp. undula*, *Vibrio rugula*, *Spiromonas Cohnii*, *Vibrio serpens*, and several species of *Bacillus*. It is only in the smallest of the bacteria that it has hitherto been impossible to demonstrate their presence. They have, however, been recently seen by Dallinger and Drysdale in *Bacterium termo*. Warming has perceived as many as two or three on one extremity in *Ophidomonas sanguinea Spirillum volutans* var. *robustum*, and *Vibrio rugula*.

PLATE I.

Taken from "Monthly Microscopical Journal," of Sept. 1st, 1875.

FIG 1. — *a. B. termo* magnified with the same power as *b*, which is a specimen of *Spirillum volutans*, showing flagella at each end.

FIG. 2. — *B. termo*, as seen with a power of about 600 diameters.

FIG. 3. — The same as seen with $\frac{1}{50}$ and second eye-piece (3,700 diameters).

FIG. 4. — *B. termo*, seen with flagellum at one end, the light coming in the direction of the arrow.

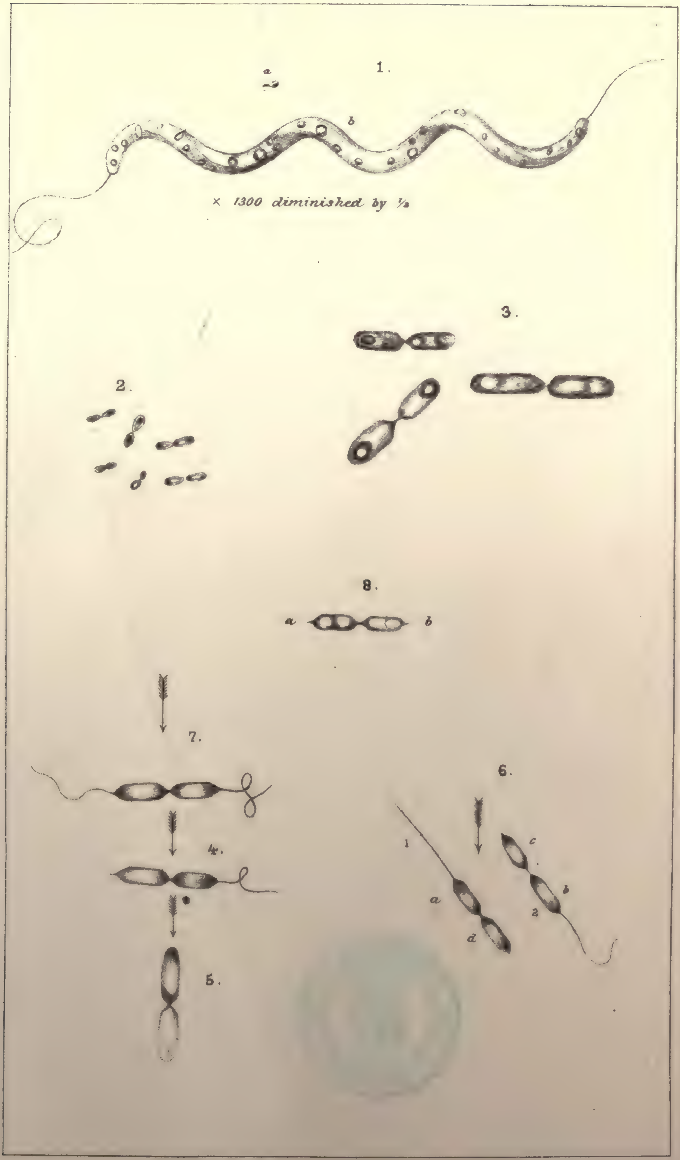
FIG. 5. — The same object when it moved at right angles to its former position, the light coming from the same direction, causing the sight of the flagellum to be lost.

FIG. 6 represents one *B. termo* which was in a still condition, but one flagellum moving. The light came in the direction of the arrow. When the end marked 2 *b* was in focus, a flagellum was seen, but none at the end *c*. When the end marked 1.*a* was focused carefully, the flagellum at that end was seen, and lost at the end *d*.

FIG. 7. — The true form of *B. termo*.

FIG. 8. — The form as shown by the "supplementary stage" illumination *before* flagella were found, showing the pointed termination of the body at *a*; *b*.

The Monthly Microscopical Journal Sept. 1. 1875. Plate I.


1.
a
b
× 1300 diminished by ½
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8.
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b
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1
a
d
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A Meisel, lith.

FLAGELLA ON BACTERIUM TERMO.

By W. H. Dallinger, F.R.M.S. and J. J. Drysdale, M.D. F.R.M.S.

EXTRACT FROM PAPER "ON THE EXISTENCE OF FLAGELLA IN BACTERIUM TERMO," BY W. H. DALLINGER, F.R.M.S., AND J. J. DRYSDALE, M.D., F.R.M.S.

"In the summer of 1872, some very fine specimens of *S. volutans* came under our notice, and were carefully examined. We were enabled fully to confirm Cohn's discovery, and demonstrated repeatedly the presence of a pair of swiftly lashing flagella. The drawing at *b*, *Fig.* 1, was made from a specimen magnified 1,300 diameters (diminished by $\frac{1}{2}$).

"Having closed for the present our Monad researches, we have been stimulated by the hope that the experience gained by these might enable us to prosecute similar investigations into the true life history of bacteria. We have commenced the work this summer, and, guided by the analogy of *S. volutans*, we have been led to make several continuous efforts to find whether or not there existed a flagellum or flagella in *B. termo.* The task, of course, under the best circumstances, must be a difficult one, from the extreme minuteness of the object. We tried each of Powell and Lealand's powers successively, from the $\frac{1}{12}$ to the $\frac{1}{50}$, but with no definite result. Repeatedly we both saw vortical action at both the distal and proximal end of the *termo*, but could not absolutely see the organ causing it. But in the process of our investigations we made very close and careful observations on the *fission* of this form: we do not purpose now to describe the process, but merely to point out a phenomenon that further confirmed our suspicion of the presence of an invisible filament. In separating into two, the jointed rod of sarcode which is in process of division shakes to and fro at the constriction, as if the constricted part were a hinge; and at length a clear separation takes place to quite the length of the original *termo* (sometimes longer), and there is no *visible connection between them;* nevertheless *they act as one creature, so that if one moves in any direction, the other goes with it, just as the two parts did before separation;* showing that, although we cannot *see* the connection, there must be one; and the presumption was that it was a fine filament, such as we detected in the fission of some monads.[1] We could make no further progress in the question apparently; but our attention was called to the new $\frac{1}{8}$th objective prepared by Messrs. Powell and Lealand, with which we were soon supplied. We used it at first with the 'supplementary stage' for very oblique illumination, supplied by the same makers, and this has the advantage of throwing the light in only from one direction. We were soon convinced of the exquisite performance of the glass when used as an immersion. *Amphipleura pellucida* was not merely seen to be striated clearly and sharply, but the striæ were resolved into beads with the third and fourth eye-pieces. In like manner the fine striæ in *Surirella gemma* were instantly shown to be beaded, with perfect and brilliant definition, with the second eye-piece. *Navicula rhomboides* and an extremely delicate specimen of *Pleurosigma attenuatum* which had resisted everything below a $\frac{1}{16}$th immersion, showed beaded striæ perfectly. We were therefore encouraged to try again to discover flagella in the *termo*. Some of our specimens, nourished in Cohn's nutritive fluid, were placed in a drop of distilled water, and put upon the supplementary stage on an ordinary slide covered with the thinnest cover. The utmost delicacy and tact in manipulation of the light is the great desideratum; but, with this, enough may be secured to work with the fourth eye-piece. The light may be made to enter the objective at almost every angle, but it is always projected in a direction at right angles to the stage; and the first thing we observed when the objects were sufficiently slow in their movements, and at right angles to the light, was that the ends of the *termo*, which we (and all other observers, as far as we know) had taken for round, proved themselves to be conical, terminating in a sharp point. The usual appearance of *B. termo*, as seen with a magnification of about 600 diameters, is seen in *Fig.* 2; whilst the same seen with a magnifying power of 3,700 diameters ($\frac{1}{50}$th and second eye-piece) is seen in *Fig.* 3, where a globular granule is seen in the end of each half. But with the method above referred to, the best conditions being secured, the two ends of the bacterium were distinctly *pointed*, as seen at *a b*, *Fig.* 8, and after nearly five hours of incessant endeavor a flagellum was distinctly seen at one end of each of two *termos* which were moving slowly across the field. The discovery was not sudden and transient, but lasted for at least twenty minutes. The exquisitely delicate flagellum was lashing rapidly the whole time; and one of its frequent conditions is shown in *Fig.* 4, the arrow indicating the direction of the light: but if the *termo* turned round at right angles, as in *Fig.* 5, all trace of the flagellum was gone, showing that its discovery depended entirely, all things being equal, upon its position in regard to the light.

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[1] "M. M. J., vol. x., p. 55; and vol. xi., p. 8."

"But this observation was made only by *one* of us, the other not being present; and in pursuance of our plan we determined to see it again, convincing ourselves separately, and then together. After many hours of labor, this was accomplished; and *Fig.* 6 shows one of two instances which we both saw together at the same time and in the same instrument. It was lying still, obliquely across the field, the light coming in the direction of the arrow. Both ends were not perfectly in focus at the same time, but in focusing the end marked 2 *b* (*Fig.* 6) the flagellum was distinctly seen, and was seen also to coil and lash; but no flagellum was then seen at the end *c* of the same object; but by bringing it into delicate focus it presented the aspect seen at 1 *a* (*Fig.* 6), which really represents the same object at the same time, only with the other end in the focus, while the end marked *d* corresponding to 2 *b* of *Fig.* 6 was in its turn slightly out of focus, and the flagellum lost to view. This observation, made together, was as satisfactory as could be desired; and it was thus demonstrated that there was a flagellum at *both ends* of the ordinary *B. termo.*

"It will of course be understood that it is by no means an easy matter to secure the demonstration; and whenever we repeat it, it must always be with nearly the same amount of trouble and patience, although we can now with the ordinary condenser detect the vortical action, both in front and (occasionally) behind the *termo,* as we never did before. But the demonstration of the ultimate structure of a fixed object — as for instance *Amphipleura pellucida* — must be looked upon as a matter of great ease in comparison; and that for many reasons, the foremost being the motion and the minuteness of the object, the swift play of the flagella, their similarity in optical properties to the fluid in which bacteria live, the difficulty of retaining them in focus, and of getting them in such a position in relation to the light as to make demonstration possible. Of course, all this would be removed if dead bacteria would answer, but they very rarely (indeed only once) have done so with us. The flagellum needs to be in slow motion to properly show itself; for even with the more delicate and minute monads it is a difficult thing to show the flagella in dead forms, since in the majority of cases they appear to be attracted round the body of the creature."

§ 2. — Of the Different Modes of Grouping of the Bacteria.

The bacteria are found in different media in two states, — free, isolated (unicellular bacteria), or united several together, either in chains, in filaments, or in masses of greater or less extent, and sometimes by the aid of a mucous substance in which they are imbedded.

The free unicellular bacteria are found in the *Spirillum, Bacillus, Monas,* etc. When they are united, they are grouped in the following modes: —

1. *Form of a little chain: Torula, Leptothrix.* — The usual method of multiplication among the bacteria is by *fission* ("scissiparité"); each corpuscle divides transversely, and gives birth to two

new individuals, which sometimes become separated completely the one from the other, to form unicellular bacteria, sometimes remain united; and segmentation again occurring in each portion, a chain is formed of articles more or less numerous.

When these chains are formed of spherical bacteria, they have been called *torulæ;* if they are formed of filiform bacteria, they correspond to *leptothrix* (Robin). The morphological difference between the *torula* and the *leptothrix* consists in the fact that in the first the articles are separated by constrictions, while this is not the case in the second. It is also to be remarked, according to Cohn, that the microbacteria never take either of these forms. Warming states, however, that he has met the *torula* form in *Bacterium lineola*, *B. catenula*, and *B. termo* (?).

Billroth has called these two forms of bacteria *streptococcos* and *streptobacteria.* He has even considered it necessary to create the words *diplococcos* and *diplobacteria* for organisms constituted only of two articles.

2. *Form of Zooglœa.* — Generally, when bacteria are rapidly multiplying, they remain grouped in masses, swarms, or *Zooglœa.* In the latter condition, they are closely pressed against each other in the midst of a viscous substance, hyaline, homogeneous, colorless, and constituting masses more or less diffused or definite, in irregular globes, bunches, or tubes, swimming in the water or near its surface. When the bacteria multiply abundantly, the cells become removed from each

other, so as to leave between them greater intervals. The masses sometimes attain a diameter of several centimetres.

The gelatinous substance in which the bacteria are included seems to be produced by a thickening and jellification of this cell-membrane, or by a secretion from their protoplasm, but the latter view seems more plausible than the former (De Lanessan).

It is commonly the spherical bacteria (*Micrococcus*) and the microbacteria (*Bacterium*) which are found in this state.

The filiform bacteria and the spirilla are never found in gelatinous masses (Cohn). Ray-Lankester, however, claims to have met the *Spirillum tenue*, in the form of zooglœa, and Klein the *Spirillum undula* and *rosaceum* (Warming).

The form of *Zooglœa*, properly so called, gelatinous and thick, has never been found by Warming in infusions of sea-water.

According to the terminology of Billroth the zooglœa are called *gliacoccos* and *gliabacteria* (from *γλία*, mucus substance).

3. *Form of Mycoderma.* — In certain cases, the bacteria unite on the surface of the water, or of the liquid in which they are developed, to form a thick layer, a sort of membrane. This production called *mycoderma* by Pasteur is a sort of *zooglœa*, but differs from it by the absence of the intermediary mucous substance. The bacteria are, however, motionless, although living, since they come to the surface to be in contact with oxygen, which is necessary to them.

The *petalococcos* and *petalobacteria* of Billroth correspond with the *mycoderma* of Pasteur.

4. *Swarms.* — We have seen that the filiform and spiral bacteria do not, usually, form *zooglœa.* These microphytes are either disseminated and free, or united in swarms. This formation may be seen, for that matter, in all the bacteria, when, thanks to abundant nourishment, they multiply rapidly and gather together in considerable masses. They are very active in these swarms, whilst in the *zooglœa* the corpuscles are motionless, because of the intermediary glairy substance.

Pulverulent precipitate. — When the nutritive elements are exhausted in a liquid, the bacteria cease to multiply, fall to the bottom of the receptacle, and the liquid gradually becomes clear. The deposit formed in this manner may acquire a thickness very appreciable to the naked eye. The bacteria which form this precipitate are not dead, but in a state of temporary repose; and if a new supply of nutritive material is added to the liquid, they are seen to multiply anew, until this has been exhausted (Cohn).

PLATE II.

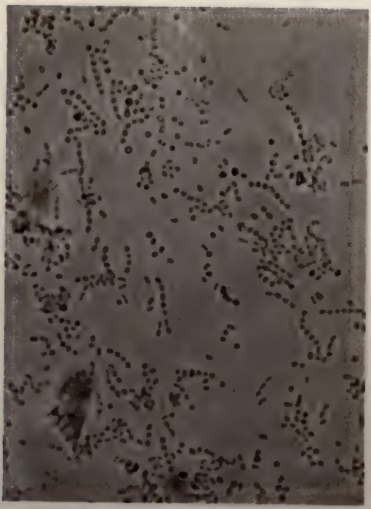

FIG. 1.

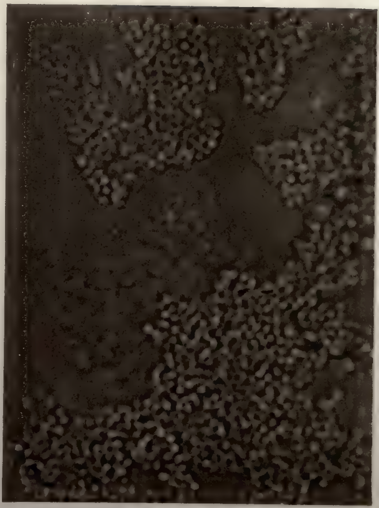

FIG. 2.

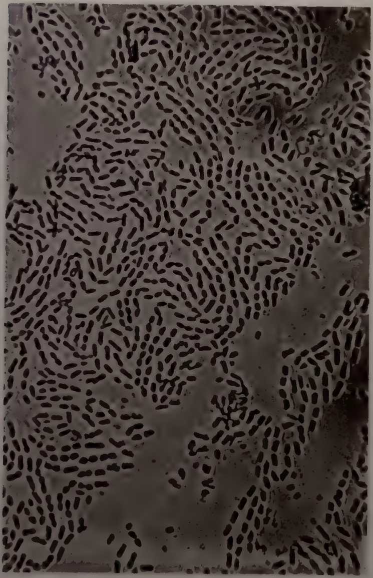

FIG. 3.

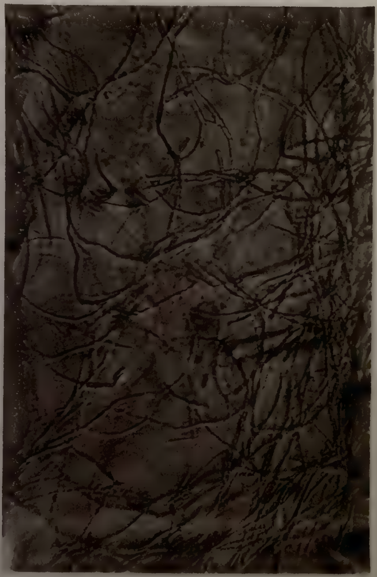

FIG. 4.

PLATE II.

DIFFERENT MODES OF GROUPING.

From photo-micographs made in Havana and New Orleans; copied by permission of the National Board of Health.

FIG. 1. — *Torula form* of spherical bacteria (*Micoderma aceti* Pasteur) from rotten banana, New Orleans, April, 1880. × 1000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIG. 2. — *Zooglœa form* of spherical bacteria developed in culture-cell containing blood of leper. × 600 diameters.

FIG. 3. — *Mycoderma*, from surface of foul gutter-water. New Orleans, April, 1880. × 400 diameters by Beck's $\frac{1}{5}$ in. objective.

FIG. 4. — *Leptothrix form* of desmobacteria developed in yellow-fever urine, exposed in laboratory, Havana, July, 1879. × 400 diameters by Beck's $\frac{1}{5}$ in. objective.

CHAPTER II.

CLASSIFICATION OF THE BACTERIA.

§ 1. — POSITION OF THE BACTERIA.

THE place of the bacteria in the scale of beings, for a long time undetermined, demands to be established with precision; not only for the naturalists, who only view the question from a systematic point of view, but above all for the biologists who study the *rôle* of these organisms in the chemical or pathological phenomena with which they are associated. According to Ch. Robin, not to define the animal or vegetable nature of these organisms, " is for them as grave as it would be for a chemist to leave undecided the question as to whether it was nitrogen or hydrogen, urea or stearine, which he had obtained from a tissue, or of which he is following the combinations in certain operations."

This determination is, to-day, possible; and, if there are still some differences of opinion among naturalists as to the place of the bacteria among the cryptogams, there is but one opinion as to their vegetable nature.

It is surprising to see a *savant* like M. Pasteur " not to pronounce positively upon the vegetable or animal nature of several of the ferments which he has studied," and of which some belong to the bacteria.

We shall first indicate rapidly the characters which permit us, at first, to recognize certain species of bacteria as organized beings, to determine if they are animal or vegetable, and finally to classify them either among the algæ or among the fungi.

Distinction of Bacteria from Inorganic Substances. — The question as to whether bacteria are organized beings can only be raised in relation to the smallest species, those *Micrococci* which are scarcely perceptible with the highest powers; the organized nature of the other organisms of the same group has never been questioned, even by the earliest observers, who all, since Leeunhoeck, have, without exception, taken them for animals or vegetables. But the smallest forms of bacteria may be confounded with various matters, with organic particles, molecular granules, fat globules, etc. " These productions, which are found in considerable quantity in the liquids or in the tissues of animal or vegetable origin, often resemble so closely, in form, size, and grouping, the spherical bacteria, that it is absolutely impossible to guard one's self against confusion, unless the most minute precautions are taken in making the observations" (Cohn).

The *detritus*, the amorphous powder of precipitated molecules of inorganic substances, even when they exhibit the *brownien* movement, are easily enough distinguished from *Micrococci* by optical signs, their angular form, their less refractive power, and finally by their reaction with certain chemical agents; above all if they are mineral substances, crystalline bodies, etc.

It will not be the same with molecular granules of organic nature. They have as common characters, their rounded form, their notable refractive power, movements. Nevertheless, their form is less regular, more angular, their color variable, their refractive power always less. In doubtful cases, Tiegel has given a method which enables us to distinguish them from *Micrococci*. It consists in warming the glass slide which supports the corpuscles under examination; if they are "*Coccos*," they are seen to move in a manifest manner. This does not occur in the case of molecular granules.

It is these productions which render it very difficult to observe the phenomena which occur during the coagulation of milk. The caseine separates in the form of extremely minute globules having a very rapid molecular movement. But we may distinguish these from bacteria by the use of liquor potassæ, which dissolves the former without attacking the latter.

As another example of *pseudobacteria*, I will mention, after Cohn, the form which fibrine assumes when it separates from the plasma of the blood. It disposes itself in very slender filaments, closely resembling filamentous bacteria.

Fat globules, which are found of all sizes, are often of the same dimensions as *Micrococcus*, and are very difficult to distinguish from the latter. The difference in refractive power is slight, and the action of re-agents, such as ether, is not certain in mucilaginous solutions. Hiller, who has paid especial attention to the means of recognizing bacteria, divides them into two groups:—

A. *The optical signs:* comprising 1. The characteristic vegetable form, rods, *leptothrix,* this he recognizes as of little use, as in this case there is no doubt; 2. The characteristic movements of the monads; 3. The mode of growth and of multiplication; 4. The mode of junction of the granules.

B. *The chemical signs*: 1. False *zooglœa* become softened and diffluent under the action of liq. potassæ, and are coagulated by the direct application of alcohol; 2. In sections of tissues, after an hour of maceration in liq. potassæ, diluted $\frac{1}{10}$th, the monads are colored brown by iodine, while fat granules are not.

But, in truth, the method of cultivation, extolled by Cohn and Wolff, is the best means of distinguishing the bacteria. "The distinction of pseudobacteria," says the first of these authors, "from veritable globular bacteria is a problem which our microscopists cannot resolve, in every case, with the desirable certainty. It is only by a study of their mode of development that this distinction can be made. *The globules which divide and develop in form of chains are organized beings; when this does not occur, we are dealing with pseudobacteria.*"

This is not, however, exactly the opinion of Nägeli, who seems to consider movement as the surest distinctive characteristic.

"There are," he says, "but three distinctive signs which enable us to recognize with some certainty that granules under observation are organisms, — spontaneous movement, multiplication, and equality of dimensions, united with regularity of form.

"The most certain character is movement in a straight or curved line, — a movement which inorganic granules never present. One should take care not to be deceived by movements which are caused by currents in the liquid under observation. Nor should one allow himself to be deceived by the tremulous motion, called molecular movement, in which the granules do not really change their position. These movements are seen in most cells, and even in those of the Schizomycetes, and inorganic bodies themselves present it.

"Multiplication is a character less important than movement. When among granules some are found united in pairs, it may be supposed with probability that division and multiplication are taking place. When rods are bent at an angle, one may predict their division in two parts.

"Finally, as to size and form. Granules of different size and of a more or less irregular form ought not to be considered as belonging to the group of segmented fungi; if, on the contrary, the granules offer dimensions perfectly equal, and a spherical or oval form, the distinction is more uncertain: they may belong to the schizomycetes or be of inorganic nature."

Place of the bacteria among organized beings. Distinction between animals and vegetables. — The characters which serve to distinguish the inferior animal organisms from the inferior vegetable organisms are of two orders, optical and chemical.

A. The optical characters are drawn from the general form, the movements, and the mode of reproduction.

The morphological characters have no value except among the larger species of bacteria. If we bring together a *Spirillum* and a *Spirulina*, Kütz., their affinities will be apparent to every one. It is not the same for the large species of *Bacillus*, of which the relations with the *Oscillatoria* are evident. The rod form seems very special, but it does not necessarily imply the vegetable nature of the organisms which possess it. Finally, the spherical bacteria, — *Monas* and *Micrococcus*, — resemble entirely by their form some infusorial animals.

Movement is not a more special character. It is now well proved that it does not belong exclusively to animals, and that it is met with in a certain number of the inferior vegetables.

In fact, the anatomical characters are not always absolutely reliable; but it is from these alone that Cohn first, then Davaine, have recognized the bacteria as vegetables.

B. Chemical characters. Robin depends upon these characters to demonstrate the vegetable nature of the bacteria. He takes for point of departure the notions of general physiology as given by De Blainville in the following points: —

1. We find in animals various elementary substances of the same kind as in plants, and reciprocally.

2. The ternary compounds predominate, however, in plants; and the quarternary, nitrogenized, are more abundant, on the contrary, in animals.

3. In both, the fundamental cellular structure is the same; at least originally for the greater number, and always in the most simple of organized beings, etc. . . .

"It results from this, then," continues M. Robin, "that so long as there is no digestive tube one can only distinguish plants from animals by the study of their elementary principles, and of the chemical reactions which these exhibit in general; by the study, in particular, of the reactions which the predominance of ternary cellulose principles over all others gives to plants, and that of nitrogenized principles in animals, at all periods of their existence."

Starting from this basis, Robin made numerous attempts to find in liquor ammonia, concentrated, as prepared for use in laboratories, a reagent for corpuscles of a vegetable nature. In effect, ammonia dissolves the eggs, the embryos, of all animals, the bodies of all the inferior infusoria, attacks the spermatozoa, etc., whilst it leaves absolutely intact all the varieties of cellulose and the anatomical reproductive elements of plants, whether it is used cold or boiling.

As to the other chemical characters praised during recent years, we will content ourselves with mentioning *concentrated acetic acid*, which causes all animal tissues to become pale, whilst it is without action on bacteria (Luckonvsky); iodine, and sulphuric acid (Letzerich), etc.

Hematoxyline (Luckonvsky) and fuchsin (Hoffmann) color the bacteria deeply. One ought, then, no longer to give to the bacteria, as do some recent authors, the names of microscopic animalcules, — infusoria, microzoa, etc., and other expressions without precision, or consecrating an error.

Let us add that some naturalists of high repute, Häckel for example, have created for these minute beings, monera, protoplasts, flagellata, diatoms, etc., an intermediary kingdom between the animal and vegetable, — the *Protista.*

Place of the Bacteria in the Vegetable Series. — The vegetable nature of the bacteria once established, it remains now to determine to what class of vegetables they belong.

Are they algæ, or are they fungi? This is the question which divides the naturalists.

It is true that it is to-day very difficult to find a characteristic of these two classes of vegetables, both having, in a general manner, identical forms, similar reproductive apparatus, etc.; and, if it is impossible to confound a Basidiomycete with a Florideæ, for example, it is not the same when one studies the inferior species. The only character which appears general is the presence of chlorophyll in the algæ and its absence in the fungi. But, if we adopt this distinctive character, and apply it in all its rigor, we are obliged to separate in the inferior algæ some forms very nearly related, and which do not differ from their relations except in this particular. And this is exactly what happens in the case of the bacteria.

In truth, the bacteria, although entirely without chlorophyll, have numerous affinities as to form, movement, etc., with the *oscillatoriaceæ*, and, according as one or the other of these characters have appeared to predominate, the bacteria have been classed as algæ or as fungi.

It is thus that Davaine, Rabenhorst, then Cohn, struck above all by the resemblance of form, mode of grouping, and of multiplication, have placed the bacteria among the algæ. Cohn insists, above all, upon the affinities of the filiform bacteria with the *beggiatoa* and the *leptothrix*; of the *micrococcus*, and of the *bacterium*, with the *chroococcaceæ*. He at first placed them at the commencement of this last series; but we shall see further on that in his last publications he has disseminated them among the oscillatoriaceæ and the chroococcaceæ.

Robin and Nägeli, on the other hand, insist rather upon the affinities of the bacteria with the yeast plants, which are incontestably fungi, and they include them in this class.

Robin says expressly: "All the corpuscles described under the name of *Bacterium termo*, *B.*

punctum, etc., *Zooglœa*, *Micrococcus*, and many others, are vegetable cells, spores of fungi, of several distinct species certainly; spores, or reproductive bodies of the first order, derived one from another, either by germination, fission, or from a mycelium; reproductive bodies, in a word, of the order of those which Tulasne has arranged under the name of conidia, etc."

Nägeli establishes in the *inferior fungi which produce decompositions* three very natural groups.

1. The *Mucorini*, or mould fungi;
2. The *Saccharomycetes*, or budding fungi, which produce the fermentation of wine, beer, etc.;
3. The *Schizomycetes*, or fission fungi, which produce putrefactive processes. This last group is formed of our bacteria (*Micrococcus*, *Bacterium*), etc.

Sachs solves the question by uniting the algæ and fungi in a single group, the *thallophytes*, in which he establishes two series exactly parallel,—one comprising the forms with chlorophyll; the other, the forms which are deprived of it, and preserving in a transverse direction the morphological affinities of these organisms.

As this classification is yet but little known, we think it best to give it in the following table:—

THALLOPHYTES.

Forms with chlorophyll.	*Forms without chlorophyll.*
Cl. 1. Protophytes.	
A. Cyanophyceæ (Oscillatoriaceæ, etc.).	A'. Schizomycetes (Bacteria).
B. Palmellaceæ.	B'. Saccharomycetes (Ferments).

PLATE III.

Saccharomycetes and Schizomycetes (Nägeli), developed in urine (of yellow-fever patient) exposed in laboratory of the Yellow-fever Commission, Havana, July, 1879. *Reproduced by permission of the National Board of Health.*

FIG. 1. — Photo-micrograph made with Beck's $\frac{1}{8}$-in. objective and Tolles's amplifier. 400 diameters.

FIG. 2. — Photo-micrograph made with Zeiss's $\frac{1}{18}$-in. hom. im. objective. 1,450 diameters.

PLATE III.

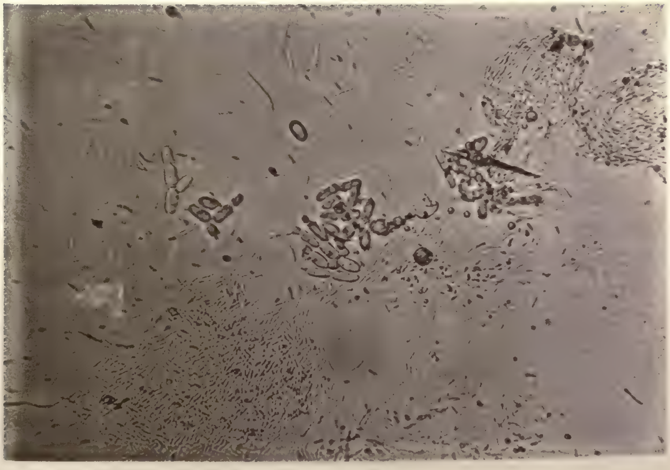

FIG. 1.

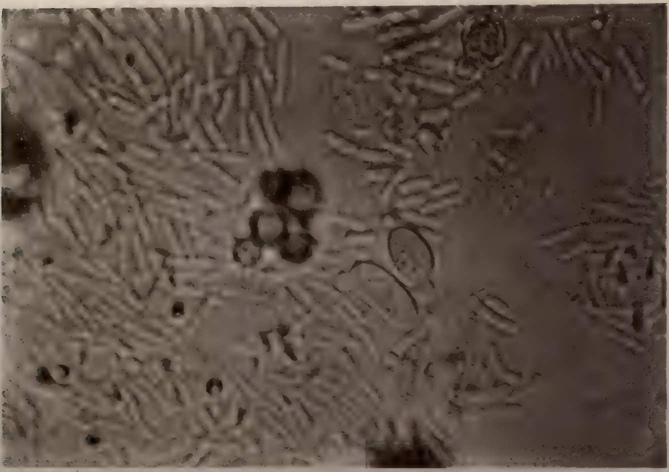

FIG. 2.

Heliotype Printing Co., Boston.

Cl. 2. Zygosporeæ.

A. Volvocineæ.	A'. Myxomycetes.
B. Conjugueæ and Diatoms.	B'. Zygomycetes.

Cl. 3. Oosporeæ.

A. Sphæropleæ.	
B. Cœloplasteæ.	Saprolegniæ.
C. Œdogoniæ.	Peronosporeæ.

Cl. 4. Carposporeæ.

A. Coleochæteæ.	A'. Ascomycetes.
B. Florideæ.	B'. Œcidiomycetes.
C. Characeæ.	C'. Basidiomycetes.

Our preferences are for this last mode of classification, but obliged, in the description of species, to follow the classification of Cohn, the most complete which has been given hitherto, we must abandon it for the present.

§ 2. — Classification; Generic and Specific Characters.

The numerous classifications of the bacteria of which we have given an abstract in the historical part of this work, show how variable have been the ideas of the microscopists as to the nature of these organisms.

Before giving the most recent, those among which we will have to choose, it is best to study the characters upon which authors have depended for grouping the bacteria in genera and species, and to estimate the value of these characters.

1. *Generic and specific characters.*— These have been drawn from the dimensions, form, movement and evolution of the bacteria.

The size, which, according to Cohn, is the dominating distinctive character, is often indeterminable, even in employing the highest powers. Besides, for a great number of neighboring forms, the differences of measurement given as distinctive are so slight that they cannot serve in practice. Thus, according to Dujardin, the *Bacterium termo* has a length of 1.7 μ, and the *B. punctum* of 1.7 to 0.6 μ. Another difficulty presents itself when we examine bacteria formed of several articles. Shall we consider the length of a single article or of the chain, which consists of a number of articles, a number ordinarily variable?

The *form* of the bacteria and their union in colonies, also offer differences, which have been utilized; but do they depend upon differences truly specific, or do they come from foreign influences, from phases of development of the same organism? Even when one uses these as distinctive specific characters, the form is sometimes of little assistance; since if one refers to the descriptions of Dujardin, the *Bacterium termo* will be found to have a cylindrical body swollen in the middle, and the *B. punctum* an elongated ovoid body.

As to movement, we have seen that the phenomena of mobility or of immobility sometimes present themselves in the same species, according to age or changes in the medium.

We have left, the mode of development, the phenomena of reproduction by fission or by spores, as the only character which can serve to establish our natural genera; but, unfortunately, this has only been ascertained for a small number of bacteria, the *Bacillus anthracis*, for example.

The genera of bacteria cannot have the same significance as among animals and superior vegetables; they can only be established in accordance with the most prominent characters, reserving the feeble modifications of these generic forms as specific characters.

Are there distinct, well-defined, species among the Bacteria?

The microscopists have given the most diverse opinions upon this subject. Müller, Ehrenberg, Dujardin, Davaine, have admitted the specific distinction of the numerous *vibrioniens* which they have described. Davaine, however, raises some doubts as to the absolute value of the species established in his time. "Those which are described to-day by the classifiers," he says, "ought to be considered as the expression of types *under which are hidden* a certain number of distinct species."

Cohn dwells still more upon the impossibility, in which we are to-day, of distinguishing with certainty genera and species among the bacteria. However, he is convinced that the bacteria are divided into species as distinctly as the other plants and inferior organisms. It is only the imperfection of our means of observation which makes it impossible to recognize these differences. This is above all true, he says, of the spirilla, which are not only distinguished from the rod bacteria, properly so called; but which present in their species some differences as constant as any well-defined species of alga or of infusoria.

Hallier, Hoffmann, Billroth, Robin, Nägeli, etc., consider the different forms of bacteria in a very different fashion. According to them they are not autonomous species, but phases of development of one or of several species.

According to Hallier, we may see, *à propos* of the polymorphism of the bacteria, the singular transformations which he has obtained by their cultivation.

According to Billroth, the bacteria belong to a single species of plants, the *Coccobacteria septica*, with the exception of the *Spirillum* and *Spirochæta*, in regard to which Billroth is not willing to give an opinion. This view has been adopted by a certain number of microscopists, and above all by the pathologists, such as Frisch, Tiegel, etc.

Robin also admits the genetic relation of *Micrococcus*, *Vibrio*, *Bacterium* and *Leptothrix*, but considers them the distinct and successive phases in the evolution of several species: 1st. Corpuscles described under the name of *Bacterium termo*, *punctum*, etc., *Micrococcus*; 2d. Mycelial filaments, *Vibrio*, etc.; 3. *Bacteria*, *Bacteridies*, *Microbacteria*, etc.; 4th. *Leptothrix* and forms more advanced.

The opinion of Nägeli corresponds very nearly with the preceding. "As much as I am convinced," he says, "that the schizomycetes cannot be grouped in accordance with their action as ferments and their exterior forms, and that altogether too many species have been distinguished; so, on the other hand, it seems to me very improbable that all the schizomycetes constitute a single natural species.

"I am rather inclined to suppose that there exists among them a small number of species, which have little in common with the genera and species admitted to-day, and of which each runs through a cycle of determined forms sufficiently numerous. Each of the veritable species of schizomycetes is not limited to presenting itself under the different forms of *Micrococcus*, *Bacterium*, *Vibrio*, and *Spirillum*, but can also show itself as the agent of acidification of milk, of putrefaction, and as the agent producing several maladies." However, Nägeli recognizes that it is necessary to distinguish these forms, notably those of *Micrococcus*, *Vibrio*, *Bacterium*, and *Spirillum*, without, however, losing from view the fact that the organisms thus classified have a very inconstant constitution, and pass continually from one form to another.

Finally, other *savants* such as M. Pasteur, take less account of the structural characters than of the physiological functions, regarding as a particular species every form of bacterium which is born constantly in a determined medium, or which causes a special kind of fermentation.

Nägeli opposes to this view the following objections. First, he has verified the presence, in the same decomposition, of several different forms of schizomycetes. On the other hand, in decompositions quite different, we may observe schizomycetes entirely similar as to their exterior form. Finally, we may change the mode of action of a schizomycete in subjecting it to a certain treatment. One sees that it is truly difficult to form an opinion as to the value of these *species purely physiological.*

To sum up, the characters which may be used in order to establish genera and species in the group of the bacteria are of small number and of very unequal value. Some, characters of form, of dimension, of movement, etc., are often difficult to determine, or have only a relative value; others, characters drawn from development and reproduction, are only known in so few species that they cannot be made to serve as a basis of classification.

One will not be surprised, then, to find that there is no natural classification of the bacteria, and that it is impossible for the naturalists to give one. All those that can be established are provisory, being only based upon the morphology of these organisms. Following the example of all the botanists, we will use an analogous classification, but without wishing to prejudge in any particular the genealogical relationship of the different organisms, which we shall consider as distinct genera and species.

§ 3. — CLASSIFICATION AND DESCRIPTION OF THE GENERA AND SPECIES OF THE BACTERIA.

We have seen in the historical portion of this work, *à propos* of the classifications which have been given of the bacteria, that, in 1872, M. Cohn, recognizing the numerous relations, absence of chlorophyll, mode of nutrition, etc., which make these organisms a natural family, divided them into four tribes: —

1. The *Spherobacteria*, or spherical bacteria.
2. The *Microbacteria*, or B. in short rods.
3. The *Desmobacteria*, or B. in straight filaments.
4. The *Spirobacteria*, or B. in spiral filaments.

In the spherobacteria, Cohn has only adopted one genus, the g. *Micrococcus*, of which the species are divided into three series, — the pigmentary M., or chromogenes, the M. of fermentations, or zymogenes, and the M. of contagious affections, or pathogenes.

The microbacteria include only the genus *Bacterium*, with two species, *B. termo*, Dujardin, and *B. lineola*, Cohn.

The desmobacteria comprehend the g. *Bacillus* and *Vibrio;* the first established by Cohn for the rectilinear filaments is composed of the *B. subtilis*, Cohn (with *B. anthracis* as a variety) and the *B. ulna*, Cohn; the second, characterized by undulating filaments, is reduced to *V. rugula* and *serpens*, Auct.

Finally, the spiral filaments of the spirobacteria characterize the gr. *Spirillum* and *Spirochæta*, which might be united in a single genus comprising *Sp. plicatile*, *tenue*, *undula*, and *volutans*.

Since then, Cohn, struck with the affinities which each of the preceding genera presents with several genera of oscillatoriaceæ and of chroococceæ, from which the bacteria only differ by the absence of chlorophyll, has established a class of *Schizophytes*, which includes all the inferior vegetable organisms, provided or not with chlorophyll, multiplying by fission.

We give below the complete table:—

2. *Classification of the Schizophytes, Cohn.*

TRIBE 1.—GLÆOGENES.

Cells free or united in glairy families by an intercellular substance.

A. Cells free or united by 2 or by 4:
  Cells spherical . . Chroococcus, Näg.
  Cells cylindrical . Synechococcus, Näg.

B. Cells united in glairy families, amorphous in state of repose:
  a. Cellular membrane, confounded with the intercellular substance:
    1. Cells without phycochrome, very small:
      Cells spherical . . Micrococcus, Hallier.

Cells cylindrical . Bacterium, Duj.
2. Cells with phycochrome, larger:
Cells spherical . . Aphanocapsa, Näg.
Cells cylindrical . Aphanothece, Näg.
b. Intercellular substance formed of several membranes enclosed one within the other:
Cells spherical . . Glœocapsa, Kg.
Cells cylindrical . Glœothece, Näg.

C. Cells united in glairy families of definite form:
a. Families of a single layer of cells disposed in plates:
1. Cells in fours forming a plane surface . Merismopedia, Meyen.
2. Cells without regular arrangement, forming a curved surface:
Cells spherical, families with reticulated rupture . . Clathrocystis, Henfr.
Cells cylindrical, cuneiform, families divided by constriction . . . Cœlosphærium, Näg.
b Families with several layers of cells, united in spherical corpuscles:
1. Number of cells determined:
Cells spherical, colorless, arranged in fours . . . Sarcina, Goods.

Cells cylindrical, cuneiform, with phycochrome, without regular arrangement . . . GOMPHOSPHÆRIA, Kg.

2. Number of cells very great and indeterminate:
   - Cells colorless, very small . . . . . ASCOCOCCUS, Billr.
   - Cells colored by phycochrome and larger . . POLYCYSTIS, Kg.
COCCOCHLORIS, Spr.
POLYCOCCUS, Kg.

TRIBE 2.—NEMATOGENES.

Cells disposed in filaments.

A. Filaments not branched:
- *a.* Filaments free or interlaced.
  1. Filaments cylindrical, colorless, articulations not very distinct:
     - Filaments very slender, short . . . BACILLUS, Cohn.
     - Filaments very fine, long . . . . . LEPTOTHRIX, Kg.
     - Filaments larger, long . . . . . BEGGIATOA, Trev.
  2. Filaments cylindrical, with phycochrome, articles well defined, without cellular reproduction . . . HYPHEOTHRIX, Kg.
OSCILLARIA, Bosc.

3. Filaments cylindrical, articulated, with conidia:	
Filaments colorless	Crenothrix, Cohn.
Filaments with phycochrome . . .	Chamæsiphon.
4. Filaments spiral without phycochrome:	
Filaments, short, light, sinuous .	Vibrio, Ehr.
Filaments, short, spiral, rigid . . .	Spirillum, Ehr.
Filaments, long, spiral, flexible . .	Spirochæte, Ehr.
with phycochrome:	
Filaments long, spiral, flexible . .	Spirulina, Link.
5. Filaments in chaplet:	
Filaments, without phycochrome . .	Streptococcus, Billr.
Filaments with phycochrome . . .	Anabæna, Bory. Spermosira, Kg.
6. Filament flagelliform, slender	Mastigothrix, etc.
b. Filaments united into glairy families by an intercellular substance:	
1. Filaments cylindrical, colorless	Myconostoc, Cohn.
2. Filaments cylindrical, with phycochrome	Chthonoblastus. Limnoclide, Kg.
3. Filaments in chaplet .	Nostoc, etc.
4. Filaments flagelliform, slender	Rivularia, etc.

B. Filaments with false ramification:

1. Filaments cylindrical, colorless . .	Cladothrix, Cohn. Streptothrix, Cohn.
2. Filaments cylindrical, with phycochrome	Calothrix, Ag. Scytonema, Ag.
3. Filament in chaplets .	Merizomyria, Kg.
4. Filaments flagelliform, slender towards the extremity . .	Schizosiphon, Kg. Geocyclus, Kg.

An inspection of this table shows that each of the genera of the ancient group of the bacteria has been placed beside some genus of oscillatoriaceæ, which it resembles by its organization, — *Micrococcus* and *Bacterium*, beside *Aphanothece* and *Aphanocapsa; Bacillus*, beside *Leptothrix* and *Beggiatoa; Vibrio* and *Spirillum*, beside *Spirulina.*

These affinities are undeniable, and the advantages of such a classification are manifest; but, in a work like this, we cannot think of employing it. We preserve, then, in a distinct group the schizophytes deprived of chlorophyll, which may be arranged in the four primary divisions of Cohn with the exception of *Sarcina*, *Ascococcus*, *Crenothrix*, etc., and the other genera created recently by this botanist.

Thus we will describe successively: —

1. The *Spherobacteria* of Cohn; and beside them the different *Monas* recently studied, — the *Micrococ-*

cus described by Hallier in several infectious maladies.

2. The *Microbacteria.*

3. The *Desmobacteria*, including *Bacillus*, *Leptothrix*, *Beggiatoa*, and *Crenothrix.*

4. The *Spirobacteria*, including the three genera, *Vibrio*, *Spirillum*, and *Spirochæta.*

5. Finally, we will give some account of the *Merismopedia*, *Sarcina*, *Ascococcus*, *Streptococcus*, *Myconostoc*, *Cladothrix*, and *Streptothrix.*

1. SPHEROBACTERIA, Cohn.

The spherical bacteria are characterized by their rounded or oval form, their small size, often less than 1 μ. They are ordinarily isolated, often in pairs (*diplococcus*), sometimes in a chain of several articles (*streptococcus* = *torula* of Cohn), the *mycothrix* of Hallier and Itzigsohn, or in the form of *zooglœa* when they are young and actively multiplying, and that of *mycoderma*, when they are gathered upon the surface of liquids. They have no spontaneous movement, but a simple molecular trepidation.

Functions: "The spherical bacteria are ferments, not producing putrefaction, but substitutions of another kind" (Cohn).

Obs. According to the facts observed by Koch, Cohn, Pasteur, Toussaint, upon the development of certain bacteria, it is very probable that some at least of the spherobacteria are spores of *Bacillus* or of other bacteria; at least, the *micrococci* and these spores are identical in form and aspect.

The spherobacteria include only the genus *Micrococcus.*

g. Micrococcus, Cohn (Hallier *emend.* — *Microsphæria,* Cohn, *ante*).

Cells colorless, or scarcely colored, very small, globular or oval, forming by transverse division filaments of two or several articles, in form of chaplet, or united in numerous cellular families, or in gelatinous masses, all deprived of movement.

The species are divided into three physiological groups: —

a. M. Chromogenes.
b. M. Zymogenes.
c. M. Pathogenes.

SECTION (A): MICROCOCCUS CHROMOGENES.

The pigmentary bacteria grow in the state of *Zooglœa* upon the surface of the substances which furnish them with nutriment. They are always alkaline; all are avid of oxygen; their morphological characters are identical, and one can only distinguish them by their different coloring properties.

According to Cohn, they are veritable species; for 1. Their pigments offer the greatest diversity as to chemical action and by spectroscopic analysis, etc.; 2. Each species cultivated in the most diverse media produces always the same coloring matter.

They are divided into two categories, according as the pigment is soluble or not in water.

1. *Coloring matter insoluble.*

M. Prodigiosus, Cohn (*Monas prodigiosa*, Ehrb.; — *Palmella prodigiosa*, Mont.; — *Bacteridium prodigiosum*, Schrœter).

A red gelatinous mass, pink carmine, developing upon cooked alimentary substances placed in damp air, never before cooking.

It has also been observed in *red milk*, attributed incorrectly to lesions of the teats, etc. (Cohn).

M. luteus, Cohn (*Bacteridium luteum*, Schrœter).

A yellow gelatinous mass studied by Schrœter and Cohn upon potatoes.

2. *Coloring matter, soluble.*

M. aurantiacus, Cohn (*Bacteridium auriantiacum*, Schrœter).

Little drops, or stains, more or less extended, *golden yellow*, cultivated by Schrœter, upon slices of cooked potato; by Cohn, upon hard white of egg.

M. chlorinus, Cohn.

A glairy yellowish-green pigment found upon hard white of egg, not reddened by acids, but loses its color.

M. cyaneus, Cohn (*Bacteridium cyaneum*, Schrœter).

Pigment deep blue, observed by Schrœter

upon cooked potato, and cultivated by Cohn in nutritious solutions. This coloring matter is reddened by acids, and restored to blue by alkalies, just as that which forms when lichens are macerated in presence of ammonia.

M. violaceus, Cohn (*Bacteridium violaceum*, Schrœter).

Violet-blue masses or glairy stains formed of elliptical corpuscles larger than those of *M. prodigiosus*, observed first by Dr. Schneider, then by Schrœter on cooked potato.

Later, Cohn has described the two following new species (1876), which should be included in this group:

M. Candidus, Cohn.

Stains and points *white* as snow, observed upon slices of cooked potato.

M. fulvus, Cohn.

Little *rust-colored* drops, consisting of cells, globular or united in pairs, in a tenacious intercellular substance, diameter 1.5 μ, observed by Eidam, then by Kirchner, upon horse dung.

It is also to the genus *Micrococcus* that we must refer the little globular bacteria, gifted with movement, found by Eberth in *white, yellow*, and *red perspiration*, and by Chalvet in the pus on the edges of certain wounds, but which should not be confounded with the blue color produced by a *Bacterium*.

Section (b): Micrococcus Zymogenes.

Globular bacteria producing fermentations of diverse nature.

M. crepusculum, Cohn (*Monas crepusculum*, Ehrb.).

Globular cells, colorless, developing in all infusions of animal and vegetable matter undergoing decomposition.

M. ureæ, Cohn.

Oval cells, isolated, diameter 1.5 μ (Pasteur), 1.2 to 2 μ (Cohn) or united by 2, 4, to 8 (*torula*), in a line, straight, curved, zigzag, or even in cross form. In urine, of which it transforms the urea into carbonate of ammonia (Pasteur).

A *Torula* which appears identical with the preceding *Micrococcus*, produces the decomposition of hippuric acid into benzoic acid and glycollamine (Van Tieghem).

M. of stringy wine, etc.

Globular cells of 2 μ diameter, in chaplets, found in *stringy wine*, perhaps identical with the preceding (Pasteur).

A Torulaceæ quite similar is found in certain fermentations of tartrate of ammonia and of beer yeast, with or without the addition of carbonate of potash (Pasteur).

Section (c): Micrococcus Pathogenes.

Spherical bacteria found in affections of a contagious nature.

M. vaccinæ, Cohn (*Microsphæra Vaccinæ*, Cohn).

Very small micrococci, = 0.5 μ scarcely, isolated or united in pairs in recent vaccine virus and in the pus of variola pustules. By cultivation, chaplets of from two to eight cells may be obtained, then masses containing sixteen to thirty-two cells of 10 μ and more diameter.

The M. of vaccine virus and of variola are identical, and Cohn regards them as different races of the same species.

M. diphtheriticus, Cohn.

Granular cells, ovoid, measuring from 0.35 to to 1.1 μ, isolated or more often united in twos or in a chaplet of four to six cells; sometimes multiplying in colonies and extending themselves in all the diseased tissues, decomposing and destroying them (Œrtel).

M. septicus, Cohn (*Microsporon septicus*, Klebs).

Little rounded cells, of 0.5 μ, motionless and crowded in masses or united in chaplets, in the secretion of wounds in cases of septicemia (Klebs), in *zooglœa* in callous ulcers, in isolated cells, united *in pairs*, or in chaplets in the serum of *epidemic puerperal fever* (Waldyer), in all the tissues, vessels, etc., in cases of pyemia and septicemia.

M. bombycis, Cohn (*Mycrozyma bombycis*, Béchamp).

Cells with a diameter of 1 μ, ordinarily united in chaplets of two, three, four, five, or more, in the intestine of silkworms sick with "*la flacherie*" (*Pébrine*).

In a more recent work, Cohn (Beiträge, 1875, p. 201) gives them an oval form and a diameter of 0.5 μ at the outside.

Hallier has described many other *Micrococci* in diverse contagious or virulent affections. We will only refer to them in a summary manner:[1]—

M. of the variola of animals, Hallier.

Small M. endowed with active movement, furnished with a very delicate caudal appendage, sometimes united in the form of little elongated rods, found in pustules, spontaneous or inoculated, in the lymphatic canals and the ganglia of animals attacked with variola.

M. of rugeola, Hallier.

Very small colorless M., having often a caudal prolongation, in the sputa and blood of the sick.

M. of scarlatina, Hallier.

M., free or in colonies, on the surface or in the interior of the blood corpuscles, or in chains.

M. of epidemic diarrhœa, Hallier.

M. in intestinal matters with vibrios, cells, and monads. (?)

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[1] "It is quite probable that Hallier comprises, in part, under the name of Micrococcus the same organisms that I call spherical bacteria; but the doctrine of Hallier concerning Micrococcus, as has already been pointed out by Hoffmann and de Barry, is so covered by inexact assertions and improbable hypotheses, that it is impossible to draw any conclusions from the facts he has observed." — Cohn, Beit. II, p. 148.

M. of exanthematous typhus, Hallier.

M. relatively large, brown, having a rapid movement, sometimes in chains (*Mycothrix*), in the blood.

M. of intestinal typhus, Hallier.

M. very small, in repose in the blood; larger, endowed with active motions, and furnished with contractile appendices in the dejections.

M. of glanders, Zürn.

Cells free or attached to the blood globules, or even penetrating into their interior, sometimes in chains (*Mycothrix*) in the blood. M. in chains, very numerous, and endowed with rapid movements, in the lymphatic ganglia, the mucus of the frontal sinuses, and in the chancroid ulcers.

M. of syphilis, Hallier.

M. numerous, colorless, free or in globules, in gonorrhœa, the primitive ulcer, and the blood of persons suffering from constitutional syphilis.

MONADS.

Beside the Spherobacteria are placed the Monads, not the organisms described under this name by the older microscopists, comprising microphytes, spores, and infusorial animals, but the *Monas* as understood by botanists of the present day. Thus limited, the Monads include also, besides some microphytes related to the Spherobacteria, and differing from them by their greater dimensions, some organisms of doubtful affinities.

As in the case of the *Micrococci* it is very probable that the Monads are only the spores, or lower forms of bacteria higher in the scale. Cohn places the *Monas vinosa* of Ehrenberg with the *Clathrocystis roseopersicina,* Cohn (*Bacterium rubescens,* Ray-Lank.), considering it a spore of the latter.

Monas vinosa, Ehrb.

Cells spherical, oval, regular, of 2.5 μ, often united in pairs, formed of a pink substance with granules of a deeper color, having spontaneous movements. *Hab.*, waters containing decomposing vegetable matters (Ehrb. 1838, Ch. Morren 1841, Perty 1852, Cohn 1875).

M. Okenii, Ehrb.

Cells cylindrical; average length 7 to 15 μ (Cohn), 10 μ (Ehrb.), sometimes from 60 to 80 μ (Warming), diameter 5 μ; of a beautiful red color, having a rapid gyratory movement, with a cilium at the posterior extremity or two cilia at the two extremities. *Hab.*, stagnant water (Ehrb. 1836, Eichwald, Weiss, Cohn, etc.).

M. Warmingii, Cohn.

Cell cylindrical, pink, containing at its two rounded extremities some deep-red granules; length 15 to 20 μ, width 8 μ; movement uncertain, having a vibratile cilium. *Hab.*, brackish water on the coast of Norway (Warming).

M. gracilis, Warming.

Cells straight, cylindrical, pink, rounded at the two extremities; length 60 μ, thickness 2 μ; movement slow. *Hab.*, fresh water.

Rhabdomonas rosea, Cohn.

Cells pale pink, isolated, fusiform; eight times as long as broad, having a length of 20 to 30 μ, and a width of 3.8 to 5 μ; having a slow oscillatory movement, the pink substance containing numerous granules of darker color and vacuoles. *Hab.*, stagnant water.

Ophidomonas sanguinea, Ehrb.

Cells pale pink, spiral, rigid, movement active; thickness 3 μ, length of one turn of the spiral, 9 to 12 μ. *Hab.*, brackenish waters of Denmark (Warming).

Spiromonas Cohnii, Warming.

Cells spiral, flattened; 1½ turn of spiral, diam. 1.2 to 3.5 μ, width 1.2 to 4 μ. *Hab.*, coast of Denmark.

2. MICROBACTERIA, Cohn.

Rod-bacteria, cells cylindrical, short, having spontaneous movement.

A single genus, — *Bacterium.*

g. **Bacterium,** Duj. *emend.*

Cells cylindrical or elliptical, free or united in pairs during their division, rarely in fours, never in chains (*leptothrix* or *torula*), sometimes in *zooglœa* (differing from the Z. of spherical B. by a more abundant and firmer intercellular substance), having spontaneous movements, oscillatory and very active, especially in media rich in alimentary material and in presence of oxygen.

We might, as in the Spherobacteria, divide the rod-bacteria into three groups: 1. the bacteria of putrefaction, *B. termo*, *B. lineola*, and their varieties; 2. the Bacteria of the lactic and acetic fermentations, etc.; 3. Chromogenes, B. of colored milk and pus.

B. termo, Ehrb. 1830, Duj. (*Vibrio lineola*, Ehrb. ex. p. 1838; *Monas termo*, Müller).

Cells cylindrical, slightly swollen in the middle, isolated, sometimes united in pairs, two to five times as long as wide; length 2 to 3 μ, thickness 0.6 to 1.8 μ; movements oscillatory.

Appears at the end of a very short time in all infusions of animal and vegetable substances; multiplies with rapidity in numerous *zooglœa;* then disappears as other species, to which it serves as nutriment, are developed. According to recent observations, this bacterium has cilia (Dallinger, Drysdale, Warming). Warming has also found it in the state of *torula.*

B. termo is the veritable agent, the first cause, of putrefaction, it is the true ferment *saprogène* (Cohn).

M. Warming has recently described two allied forms:—

B. griseum, cells larger, more rounded; length 2.5 to 4 μ, thickness 1.8 to 2.5 μ. In infusions of fresh and salt water.

B. littoreum, cells elliptical or elongated, slightly rounded; length 2 to 6 μ, thickness 1.2 to 2.4 μ. Coasts of Denmark.

B. lineola, Cohn (*Vibrio lineola*, Ehrb. ex p., Duj., Müller, *V. tremulans*, Ehrb., *Bacterium triloculare*, Ehrb).

Cells cylindrical, straight, rarely a little twisted, larger than the cells of *B. termo*, isolated or united in pairs, sometimes in fours, never more; length 3.8 to 5.25 μ, thickness attains 1.25 μ; movements like those of *B. termo*, but a little more active.

Is found in various vegetable and animal infusions of fresh or salt water, often takes the form of *zooglœa* containing motionless rods in their mucus substance. Warming has met it in the form of chains composed of eight to ten cells (*torula*). Its protoplasm is dotted with refractive granules.

It is not known whether *B. lineola* constitutes a specific ferment (Cohn).

The *B. fusiform*, Warming, differs from the preceding by the form of its body, which is attenuated at the two extremities; length 2 to 5 μ, width 0.5 to 0.8 μ; plasma not punctated.

Beside these species, which have been well studied, may be placed the following, which demand new investigations: —

B. punctum, Ehrb.

Elongated rods, oval, colorless, having slow movements, oscillating, often united in pairs; length 5.2 μ, thickness 1.7 μ. Diverse infusions of animal substances.

B. catenula, Duj.

Body filiform, cylindrical, often united in three, four, or five; length 3 to 4 μ, thickness 0.4 to 0.5 μ. In fetid infusions, in typhoid fever (Coze and Feltz).

Vibrio lactic, Pasteur.

"Articles almost globular, very short, a little swollen at the extremities; length of an article, 1.6 μ, of a series, 50 μ."

This vibrio seems to resemble *B. catenula* or *B. termo*. It is developed, according to Pasteur, in sweetened liquids, where it causes the formation of lactic acid and the coagulation of the casein of milk. According to other researches, coagulation of casein results from the influence of a soluble ferment (zymase), and not from an organized ferment.

Acetic ferment (*Mycoderma aceti*, Pasteur, *Ulvina aceti*, Ktg.).

"Articles short, constricted, two to three times as long as broad; length 1.5 μ, often united in long chains, forming pellicles on the surface of a liquid."

This species is also very similar to the preceding. It must not be confounded with the *Mycoderma vini*, which may develop in the same media, but which is a fungus of the group of Saccharomycetes.

The acid fermentation of beer seems to be due to a form of *Bacterium* resembling *B. termo* (Cohn), but a little larger than the type. Cohn has found it in beer undergoing acid fermentation, beside oval saccharomyces, elliptical bacteria, having movement, often united in pairs, rarely in fours, etc.

Vibrio tartaric right (Pasteur).

Bacteria similar to those of the lactic fermentation,

PLATE IV.

From "Pasteur's Studies on Fermentation." Macmillan & Co., London, 1879.

"The engraving represents the different diseased ferments, together with some cells of alcoholic yeast, to show the relative size of these organisms."

FIG. 1 represents the ferments of *turned* beer, as it is called. These are filaments, simple or articulated into chains of different size, and having a diameter of about the thousandth part of a millimetre (about $\frac{1}{25000}$ inch). Under a very high power they are seen to be composed of many series of shorter filaments, immovable in their articulations, which are scarcely visible.

In No. 2 are given the lactic ferments of wort and beer. These are small, fine, and contracted in their middle. They are generally detached, but sometimes occur in chains of two or three. Their diameter is a little greater than that of No. 1.

In No. 3 are given the ferments of putrid wort or beer. These are mobile filaments, whose movements are more or less rapid, according to the temperature. Their diameter varies, but is for the most part greater than that of the filaments of Nos. 1 and 2. They generally appear at the commencement of fermentation, when it is slow, and are almost invariably the results of very defective working.

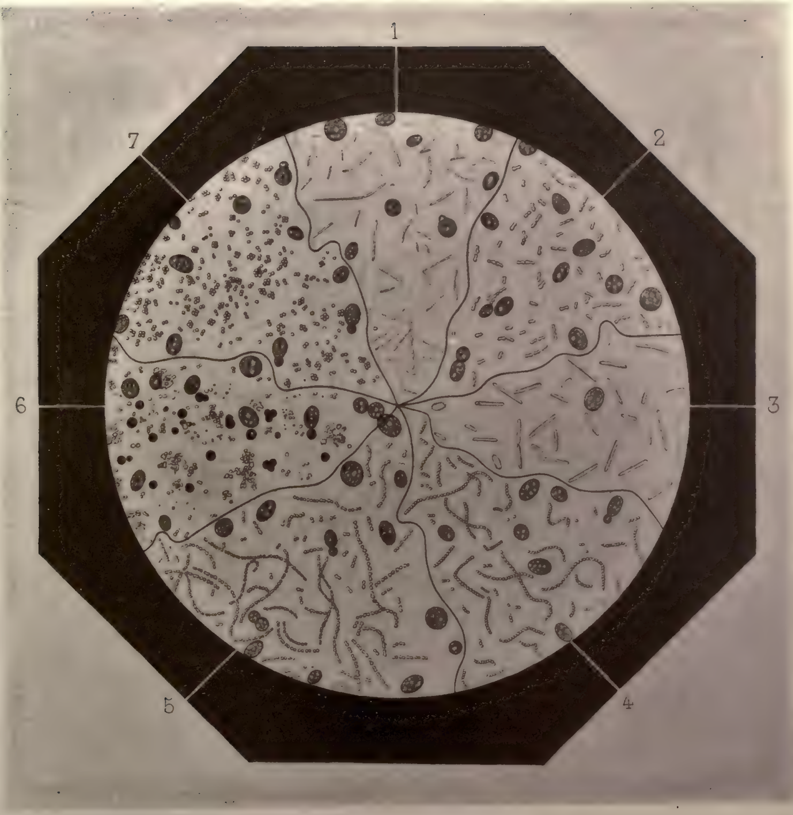
In No. 4 are given the ferments of viscous wort, and those of ropy beer, which the French call *filante*. They form chaplets of nearly spherical grains. These ferments rarely occur in wort, still less frequently in beer.

No. 5 represents the ferments of pungent, sour beer, which possesses an acetic odor. These ferments occur in the shape of chaplets, and consist of the *mycoderma aceti*, which bears a close resemblance to lactic ferments (No. 2), especially in the early stages of development. Their physiological functions are widely different, in spite of this similarity.

The ferments given in No. 7 characterize beer of a peculiar acidity, which reminds one more or less of unripe, acid fruit, with an odor *sui generis*. These ferments occur in the form of grains which resemble little spherical points, placed two together or forming squares. They are generally found with the filaments of No. 1, and are more to be feared than the latter, which cause no very great deterioration in the quality of beer, when alone. When No. 7 is present, by itself or with No. 1, the beer acquires a sour taste and smell that render it detestable. We have met with this ferment existing in beer unaccompanied by other ferments, and have been convinced of its fatal effects.

No. 6 represents one of the deposits belonging to wort. This must not be confounded with the deposits of diseased ferments. The latter are always visibly organized, whilst the former is shapeless, although it would not always be easy to decide between the two characters, if several samples of both descriptions were not present. This shapeless deposit interferes with wort during its cooling. It is generally absent from beer, because it remains in the backs or on the coolers, or it may get entangled in the yeast during fermentation, and disappear with it. Among the shapeless granules of No. 6 may be discerned little spheres of different sizes and perfect regularity. These are balls of resinous and coloring matter that are frequently found in old beer at the bottom of bottles and casks. They resemble organized products, but are nothing of the kind.

PLATE IV.



Heliotype Printing Co., Boston.

with globular articles, short; diameter 1 μ, united in chains of 50 μ.

Decomposes racemic acid, causing the right tartaric acid to disappear, and setting free left tartaric acid.

MICROBACTERIA CHROMOGENES.

B. xanthinum, Schrœter (*Vibrio synxanthus*, Ehrb.).

"Bodies cylindrical, slightly flexible, formed of corpuscles rarely exceeding five in number; length of an article, 0.7 to 1 μ. In tainted cow's milk, to which it gives a yellow color."

B. syncyanum, Schrœter (*Vibrio syncyanus*, Ehrb.).

This *Bacterium*, which has the same characters as the preceding, has been observed in sour milk, to which it gives a blue color.

B. œruginosum, Schrœter.

In greenish blue pus.

These B. chromogenes resemble entirely the lactic vibrios, *B. termo* or *catenula*. According to Robin, colored milk contains colorless vibrios, and the coloration is due to an alga similar to Leptomitus.

B. brunneum, Schrœter.

Rods in a brown coloring matter in infusions of rotten corn.

Following the colored *Microbacteria*, I place two species of *Bacterium* recently described by Ray-Lankester and Warming.

B. rubescens, Ray-Lank., 1873.

Under this name Ray-Lankester has described some phases of development of *Clathrocystis roseopersicina* of Cohn. Now Cohn is inclined to regard the *Monas vinosa*, Ehrb. as the wandering cells of *Clathrocystis*. On the other hand Warming has described his: —

B. sulfuratum, Warming, 1876, giving it for synonymes, *Monas vinosa*, Ehrb.; *M. erubescens*, Ehrb.; *M. Warmingii*, Cohn; *Rhabdomonas rosea*, Cohn. It follows, then, that the *Monas* which we have described with the *Spherobacteria* should be referred to a *Bacterium* called *sulphuratum* by Warming, but which is also identical with *B. rubescens* of Ray-Lankester.

3. DESMOBACTERIA.

Filiform bacteria, composed of elongated cylindrical articles, isolated, or in chains more or less extended, resulting from transverse division. Under this form they correspond to *leptothrix*, Auct. (differing from *torula* in that the filaments are not constricted at the point of junction of the articulations); filaments sometimes united in swarms, never in *zooglœa*. Movements and state of repose alternating and depending upon the presence or absence of oxygen, the reaction of the medium, and other conditions unknown. Some forms never exhibit movement.—*Bacteridie* of Davaine (Cohn).

We will only preserve in the *Desmobacteria* the genus *Bacillus*, Cohn. The vibrios are rather allied to *Spirillum* because of their undulating filaments.

However, after the exposition of the different species of *Bacillus*, we will say something of three genera of colorless oscillatoriaceæ, which are nearly related to them, — the *Leptothrix*, *Beggiatoa*, and *Crenothrix*.

1. Fil. with indistinct articulations	
Fil. very slender, short	BACILLUS.
Fil. very slender, long	LEPTOTHRIX.
Fil. thick, broad	BEGGIATOA.
2. Fil. articulated distinctly	CRENOTHRIX.

g. **Bacillus**, Cohn.

The *bacilli* are characterized by slender filaments, straight, short or of moderate length, rigid or flexible, endowed or not with motion.

One species is chromogene, the *B. ruber* of Cohn. Finally it is to this genus that we should refer the *Amylobacter* of Trécul.

B. subtilis, Cohn (*Vibrio-subtilis*, Ehrb.; *Ferment butyrique*, Pasteur).

Filaments very slender and elongated, formed of a single cell having usually a length of 6 μ, or of two articles of which the total length is then 12 μ, or of three (length 16 μ), or of a greater number (sometimes as many as twenty, with a total length of 40 to 60 and 130 μ); thickness not measurable, well-defined movements of flexion, active or passive, and of translation forward or backward; reproduction by fission and by globular or oval spores developing themselves in the interior of the articles (Cohn). Is found in stagnant water.

Plays a great *rôle* in the butyric fermentation (Pasteur). This B. exists in rennet, can support a temperature of 105°, and live in a medium deprived of free oxygen, in which case it takes a form *céphalée*, containing persistent spores, which, when set free, give birth to other rods of *Bacillus* (Cohn).

B. anthracis, Cohn (*Bactéridie charbonneuse*, Davaine).

Species very similiar to the preceding, generally longer and always motionless; length 4 to 12 and even 50 μ, thickness, scarcely appreciable, 0.8 to 14 μ (Bollinger).

The B. anthracis is developed in charbon (malignant pustule of man, *sang de rate* of sheep, *maladie de sang* of cattle, *fièvre charbonneuse* of horses), and in the rabbit, the rat, etc.; never in the dog, the cat, the birds, and cold-blooded animals. It is found above all in the capillary vessels. Cultivated in suitable media, such as the aqueous humor of the eye of the ox, the *Bacillus* of anthrax develops spores in the interior of its filaments, which may germinate and reproduce rods (Koch).

According to recent observations not yet published, by cultivating the *B. Anthracis* in the blood of the dog, a development of veritable sporangia may be obtained, containing from three to six spores (Toussaint).

B. amylobacter, Van Tieghem (*Amylobacter*, *Urocephalum* and *Clostridium* Trécul).

B. occurring, like the preceding, under various forms,—in pointed cylindrical filaments of 6.6 to 26 μ in length and 1.1 μ in thickness, or in form of tadpole, with a spore in the terminal swelling, or of a spindle, with a spore in the middle. In fact, it does not differ from *B. subtilis*, except by the appearance of starch in its protoplasm at the end of the period of multiplication. These B. are sometimes endowed with movement (Nylander).

It develops in vegetable tissues, which fall into putrefaction, spontaneously according to Trécul, or introduced from without by a mechanism still unknown. This is the essential agent of vegetable putrefaction (Van Tieghem).

B. ulna, Cohn (*Vibrio bacillus*, Ehrb.).

Filaments articulated, thick, and rigid, formed of one, two to four articles, straight or broken in zigzag; length of an article 10 μ, length of a filament of four articles 42 μ; slow movements of rotation and of progression.

Develops in various infusions of fresh or salt water. In certain cultivations, Cohn has seen large globules (spores?) form in the protoplasm. Warming believes that he has seen cilia.

B. ruber, Cohn.

Long rods, isolated or united in two or four, movement very active; in a red mucous substance, vermillion, developed upon grains of rice. Observed by Franck and Cohn.

Davaine has described five additional species of Bacteridies, which appear to be *bacilli*. They are:—

La Bacteridie intestinal.

Filaments straight, thick from 10 to 40 μ in length. In the intestines of birds.

La B. du levain.

Filaments slender and short, of 10 to 20 μ, divided into two, sometimes three to four articles, identical with the B. of charbon (Davaine).

La B. glaireuse.

Filaments extremely slender, straight or elbowed; attaining 10 μ in length.

La B. du vin tourné.

Filaments very slender, of variable length, flexible, indistinctly articulated.

La B. des infusions.

Filaments of 10 to 20 μ. In various infusions.

g. Leptothrix, Ktz.

The *Leptothrix* differ from *Bacilli* by their filaments being very long, adherent, very slender, and indistinctly articulated.

Their forms are numerous. The following are the principal: —

L. rigidula, Ktz.

Length 100 to 150 μ, diameter 1.3 to 1.9 μ. In stagnant water, adherent to other vegetables.

L. crœspitosa, Ktz.

Length 100 to 200 μ, diameter 2.4 to 2.8 μ. Upon humid rocks.

L. brevissima, Ktz.

Length 75 to 100 μ, diameter 2.7 to 3.5 μ. In stagnant water.

L. pusilla, Rabh.

Length 60 to 70 μ, diameter 0.5 to 0.6 μ.

L. parasitica, Ktz.

Length 90 to 150 μ, diameter 1 μ.

L. radians Ktz., and *L. spissa*, Rabh.

Parasites upon marine algæ.

g. Beggiatoa, Trev.

Filaments very slender, surrounded by mucous matter, rigid, having oscillatory movements. Protoplasm white, enclosing numerous granules, which recent observations have demonstrated to be crystalline sulphur (Cramer, Cohn).

The *Beggiatoa* are found most abundantly in thermal sulphur waters, where they constitute flocculi, which have been named *Glairine, Barégine.* They often live in water not containing free oxygen.

They play a great *rôle* in the elimination of sulphur and the disengagement of sulphuretted hydrogen in thermal waters.

Their principal species are: —

B. alba, Trev.

A whitish mucous mass enclosing colorless filaments, having a diameter of 3.5 to 4 μ. In most thermal and stagnant waters.

B. arachnoidea, Rabh.

Flocculi very minute, snow white, filaments as long as broad, — 5.4 to 7 μ. In the thermal waters of Europe.

B. nivea, Rabh.; *B. leptomitiformis*, Trev., nearly related species, living in the same conditions.

Cohn and Warming have also described: —

B. mirabilis, Cohn, articles scarcely flexible, measuring 20 to 40 μ.

B. minima, Warming, a very small species, very flexible; length 40 μ, thickness 1.8 to 2 μ.

4. SPIROBACTERIA.

This tribe includes the bacteria with undulating filaments, or filaments in spirals, more or less developed, from the *Vibrio rugula*, which only presents a single curve in its centre, to certain species of *Spirillum* which have numerous turns of the spiral. In several species, cilia, or a flagellum, have recently been observed.

We divide them into three genera:—

Fil. short, slightly sinuous . . .	VIBRIO.
Fil. short, spiral, rigid	SPIRILLUM.
Fil. long, spiral, flexible	SPIROCHÆTE.

g. **Vibrio**, Auct. *emend.*

Body filiform, more or less distinctly articulated, always undulating, having serpentine movements. This genus forms the transition between the *Desmobacteria* and *Spirillum* "from which it cannot be separated" (Warming).

Fil. thick, with a single curve . . .	V. RUGULA.
Fil. slender, with several undulations .	V. SERPENS.

V. rugula, Müller (*V. lineola*, Duj. *ex parte*).

Filament presenting in its centre a single curvature, feeble but distinct; length 8 to 16 μ. The shortest are slightly curved ($= 6\ \mu$ Warming), the larger, which may attain 17.6 μ (Cohn), 35 μ (Warm.), are about to divide. Movements of rotation more or less rapid around their longer axis; of progression forward, giving then the idea of a serpentine movement: having a cilium (Warming).

V. rugula is commonly found in swarms, in infusions, in deposits upon the teeth, in intestinal matters (Leeuwenhœck), in choleraic discharges (Pouchet).

V. serpens, Müller.

Filament one half less in diameter than the preceding, rigid, annulate, having two or three regular undulations, at least two in the shortest; height of one turn of the undulations 8 to 12 μ, diameter 1 to 3 μ, total length 11 to 25 μ, thickness 0.7 μ; movements analogous to those of *B. subtilis;* having a cilium (Warm.).

In numerous swarms in infusions, river water, etc.

g. Spirochæte, Ehrb.

S. plicatilis, Ehrb.

Filament not extensible, twisted in a long helix, *flexible*, the turns of the spiral near together; susceptible of twisting upon its axis and of an undulatory movement; total length 130 to 200 μ.

Rare species; in infusions, stagnant water, sea-water, etc.

S. Obermeieri, Cohn.

Does not differ from the preceding, either in size, conformation, or in its movements, but by its habitat and physiological peculiarities.

In the blood of persons attacked by recurrent fever (Obermeier, 1872, Weigert, Birch-Hirschfeld, etc.) during the period of access, never during the remission.

S. gigantea, Warming. Found upon the coasts of Denmark; thickness of body, 3 μ, height of spiral 25 μ, diameter 7 to 9 μ.

g. Spirillum, Ehrb.

Filament spiral, rigid; turns of spiral short and regular.

S. tenue, Ehrb.

Filament slightly tortuous, three to four turns of the spiral; length and diameter of a single turn, 2 to 3 μ. When the filament has a turn and a half, it resembles an Ω; the filaments of two to five turns have a length of 4 to 15 μ; spiral movement very rapid.

In infusions, etc.

S. undula, Ehrb. (Vibrio prolifer, Ehrb.)

Filament larger, turns of the spiral wider apart (from 3 to 5 μ); having usually one half a turn to one full turn, rarely one and a half, two, or three; length 8 to 10 μ, breadth 5 μ, thickness of filament 1.3 μ; having a very rapid spiral movement.

Fetid animal and vegetable infusions and running water.

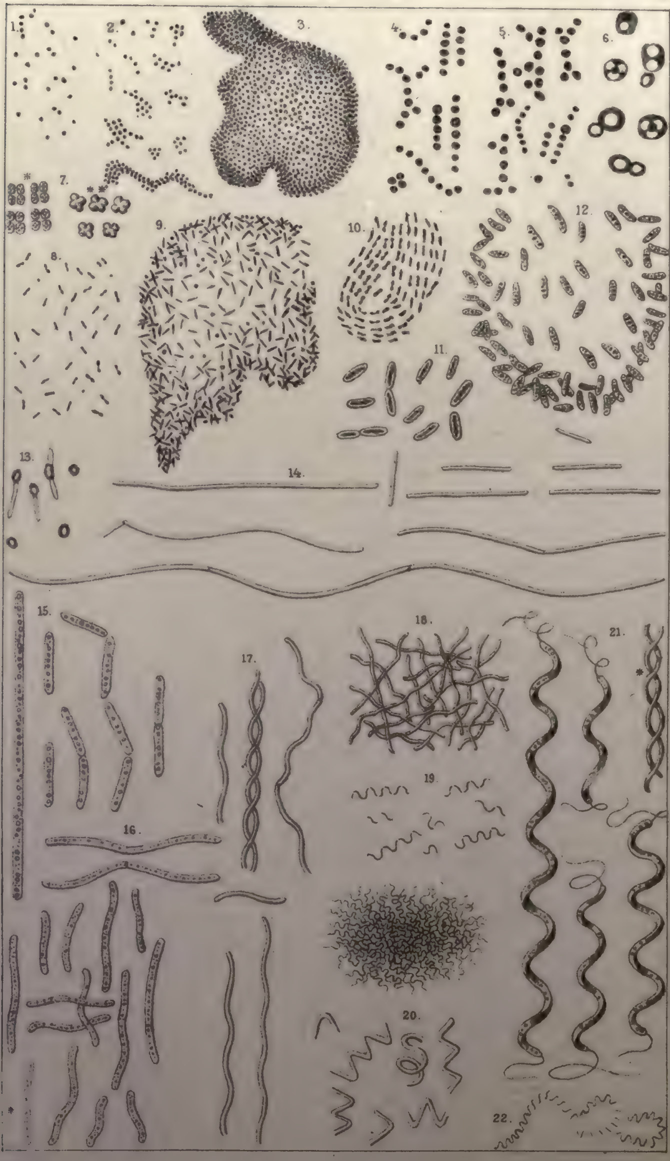
The *S. rufum*, Pertz, only differs from this by its reddish color.

S. volutans, Ehrb.

Filament large and thick, turns of spiral regular, well separated, and 13 μ in height; number of turns two, three, and three and a half, rarely six and seven; total length 25 to 30 μ, thickness 1.5 μ, breadth 6.6 μ; movement sometimes rapid, sometimes motionless; a well-defined cilium, already seen by Ehrenberg (Cohn, Warming).

This giant of the bacteria is found in vegetable and animal infusions, in sea-water, and in fresh water.

From Micr. Journ Vol. XIII, N.S. Pl.V.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A.Meisel lith.

PLATE V.

From "Microscopical Journal."

Fig. 1. — *Micrococcus prodigiosus* (*Monas prodigiosa*, Ehr.). Spherical bacteria of the red pigment, aggregated in pairs and in fours; the other pigment bacteria are not distinguishable with the microscope from this one.

Fig. 2. — *Micrococcus vaccinæ.* Spherical bacteria, from pock-lymph in a state of growth, aggregated in short four to eight-jointed straight or bent chains, and forming also irregular cell-masses.

Fig. 3. — Zooglœa-form of micrococcus, pellicles or mucous strata characterized by granule-like closely set spherules.

Fig. 4. — Rosary chain (Torula-form) of *Micrococcus ureæ*, from the urine.

Fig. 5. — Rosary-chain and yeast-like cell-masses from the white deposit of a solution of sugar of milk which had become sour.

Fig. 6. — *Saccharomyces glutinis* (*Cryptococcus glutinis*, Fersen.), a pullulating yeast which forms beautiful rose-colored patches on cooked potatoes.

Fig. 7. — Sarcina spec,* from the blood of a healthy man,** from the surface of a hen's egg grown over with *Micrococcus luteus*, forming yellow patches.

Fig. 8. — *Bacterium termo*, free motile form.

Fig. 9. — Zooglœa-form of *Bacterium termo.*

Fig. 10. — Bacterium-pellicle, formed by rod-shaped bacteria arranged one against the other in a linear fashion, from the surface of sour beer.

Fig. 11. — *Bacterium lineola*, free motile form.

Fig. 12. — Zooglœa-form of *B. lineola.*

Fig. 13. — Motile filamentous Bacteria, with a spherical, or elliptical highly refringent "head," perhaps developed from gonidia.

Fig. 14. — *Bacillus subtilis*, short cylinders and longer, very flexible motile filaments, some of which are in process of division.

Fig. 15. — *Bacillus ulna*, single segments and longer threads, some breaking up into segments.

Fig. 16. — *Vibrio rugula*, single or in process of division.

Fig. 17. — *Vibrio serpens*, longer or shorter threads, some dividing into bits, at * two threads entwined.

Fig. 18. — "Swarm" of *V. serpens*, the threads felted.

Fig. 19. — *Spirillum tenue*, single and felted into "swarms."

Fig. 20. — *Spirillum undula.*

Fig. 21. — *Spirillum volutans*,* two spirals twisted around one another.

Fig. 22. — *Spirochæte plicatilis.*

All the figures were drawn by Dr. Ferdinand Cohn with the immersion lens No. IX. of Hartnack Ocular III., representing a magnifying power of 650 diameters.

M. Warming has recently described three new species found upon the coast of Denmark: —

Sp. violaceum, height 8 to 10 μ, diameter 1 to 1.5 μ, thickness 3 to 4 μ; a cilium at each extremity.

Sp. Rosenbergii, height of helix 6 to 7.5 μ, thickness 1.5 to 2.6 μ.

Sp. attenuatum, body very attenuated at the two extremities, without a cilium.

We give below some details concerning the other colorless Schizophytes: —

g. *Sarcina*, Goods.

The *Sarcina*, which it is useless to describe here, can be considered as bacteria in which the division occurs by two perpendicular partitions in such a manner that multiplication takes place in two directions.

Sarcina is very nearly allied to *Merismopedia*, from which it only differs by the absence of chlorophyll; its siliceous skeleton allies it with the diatoms.

g. *Ascococcus*, Billr.

Cells hyaline, small, globular, closely united in globular or oval families, irregularly lobed and lobulated, surrounded by a thick gelatinous envelope, cartilaginous, forming a soft membrane, flaky, easily separating.

A. Billrothi, Cohn.

Families in masses of 20 to 160 μ, surrounded by a thick membrane of 15 μ.

In a solution of tartaric acid exposed to the air.

g. *Myconostoc*, Cohn.

Filaments very slender, colorless, folded, rolled up in a mucous substance, united in very small globules.

M. gregarium, Cohn.

Unique species found on the surface of a putrefying infusion.

g. *Cladothrix*, Cohn.

Filaments in form of *leptothrix*, very slender, colorless, not articulated, rigid or a little undulating, falsely dichotomous.

Cl. dichotoma, Cohn. In foul water.

g. *Streptothrix*, Cohn.

Filaments in form of *leptothrix*, very slender, colorless, not articulated, straight or slightly spiral, a little branched.

Str. Fœrsteri, Cohn.

In concretions in the lachrymal canal of man.

PLATE VI.

From photo-micrographs made in Havana and New Orleans. Reproduced by permission of the National Board of Health.

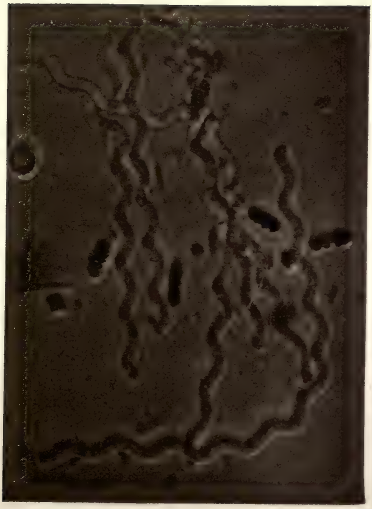
FIG. 1. — *Spirochæte* (*plicatile?*). From foul bilge-water, Havana, Sept. 1879. × 1,450 by Zeiss's $\frac{1}{18}$ in. objective.

FIG. 2. — Vibrios from water of harbor, Havana, near discharge of sewer, Aug., 1879. × 1,450 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIG. 3. — *Sarcina* (sp. ?). From standing water in flower-vase, Lafayette cemetery, New Orleans, April, 1880. × 400 diameters by Beck's $\frac{1}{5}$ in. objective.

FIG. 4. — *Spirillum* (volutans ?). From foul gutter-water, New Orleans, May, 1880. × 600 diameters.

PLATE VI.



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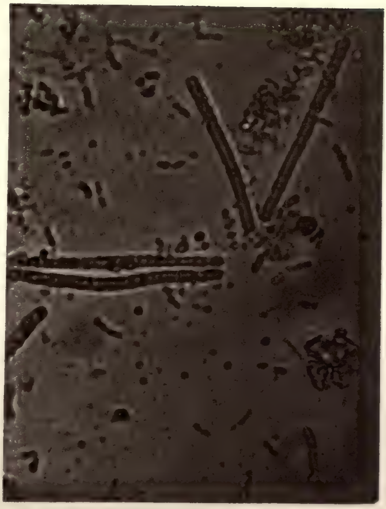

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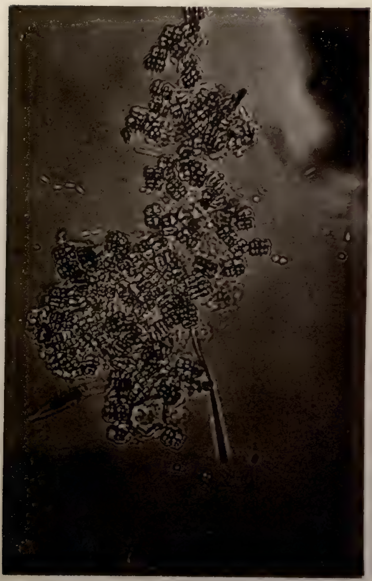

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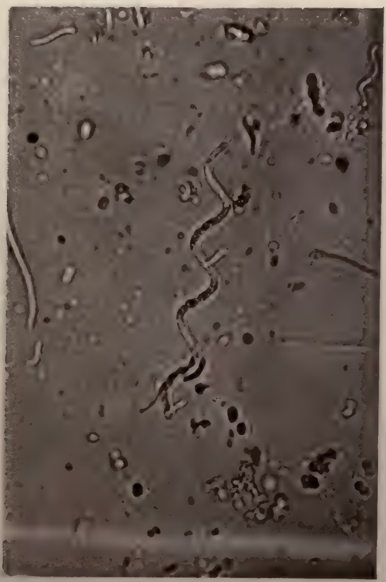


FIG. 4.

PART SECOND.

PHYSIOLOGY OF THE BACTERIA.

CHAPTER I.

DEVELOPMENT OF THE BACTERIA.

THE bacteria are now known to us from a morphological point of view: let us proceed to study the *life* of these microscopic beings; first, from a general point of view, that is to say, by studying their functions of nutrition and reproduction, independently of the special characters impressed upon these functions by certain media; then by considering the relations which are established between the bacteria and the particular media in which they may be developed.

The bacteria are of all beings the most widely diffused. We meet them everywhere, — in the air, in water, upon the surface of solid bodies, in the interior of plants and animals. If we expose a transparent liquid containing traces of organic substances, we find after a short time that it has become clouded, and the microscope shows us that it contains myriads of these beings.

What is the source of these organisms so widely disseminated, and which develop so rapidly? This

PLATE VII.

DISSEMINATION OF THE BACTERIA.

From photo-micrographs made in New Orleans. Copied by permission of the National Board of Health.

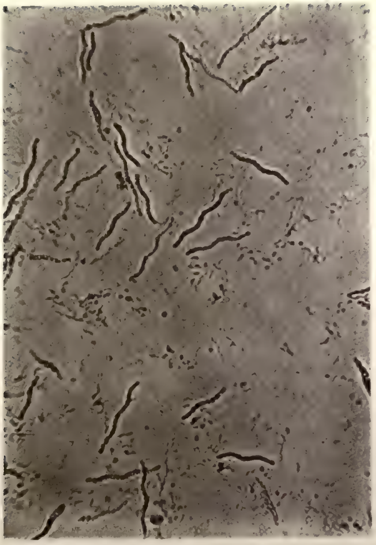
FIG. 1. — Spirillum (Sp.?) from water of salt marsh, near Salem, Mass. × 400 diameters by Beck's $\frac{1}{5}$ in. objective.

FIG. 2. — Bacteria in distilled water (see note on page 107). × 1,000 diameters by Zeiss's $\frac{1}{12}$ in. objective.

FIG. 3. — Leptothrix buccalis, epithelial cell, etc., from human mouth. × 1,000 diameters by Zeiss's $\frac{1}{12}$ in. objective.

FIG. 4. — Bacteria from human fœces. × 1,500 diameters by Zeiss's $\frac{1}{18}$ in. objective.

PLATE VII.



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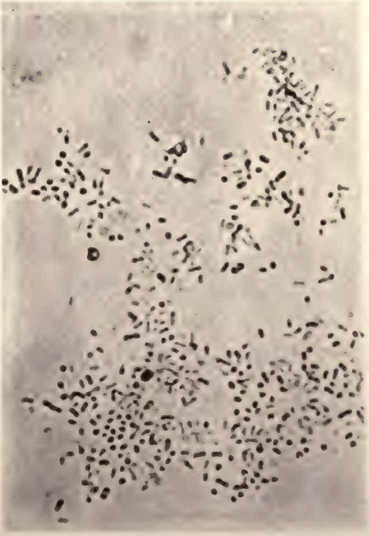
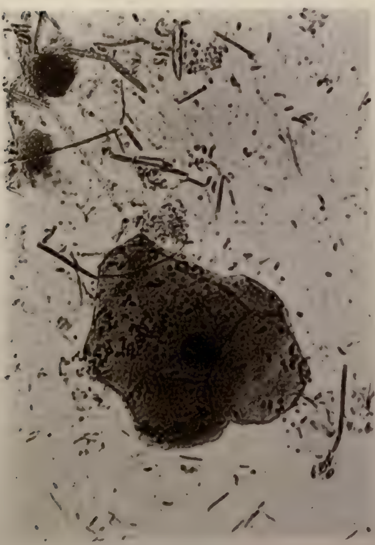


FIG. 2.



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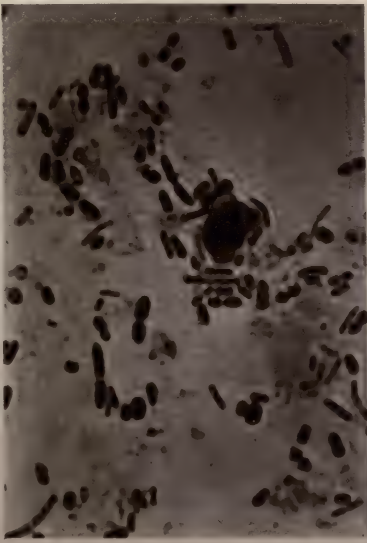

FIG. 4.

is the first question which presents itself,—a question which has given rise to long discussions, in the examination of which we shall only enter in order to give a short historical statement.

§ 1. — Origin of the Bacteria.

The origin of the bacteria, as of all the other inferior organisms, is conceived in three different manners: —

1. For some, these organisms are produced by *heterogenesis;* that is to say, by creation outright from mineral or organic substances (spontaneous generation).

2. According to others, they come directly from individuals like themselves, by one of the known modes of generation, — fission, spores, etc.

3. Finally it is believed that they are derived from organisms already existing, and are nothing more than different states or phases of development of known species, of which the life cycle is not yet discovered.

We will examine the latter hypothesis, which constitutes what is called polymorphism, when we speak of the phenomena of reproduction.

As to the two first, we will content ourselves with indicating the late works which have appeared for and against each; insisting above all upon the facts which relate to the proof of the presence of bacteria or their germs in the air, water, and liquids or tissues of the human organism, — blood, urine, etc.

Heterogenesis. — Since the experiments of Pouchet and of his pupils, and the arguments given by MM. Trécul and Frémy, the last facts invoked in favor of heterogenesis are due to MM. Onimus, Servel, Bastian, etc.

M. Onimus contends that the "proto-organisms may be born in media, protected against the air, which contain albuminoid substances."

M. Martin sustains an analogous idea. According to him, the bacteria are derived from protein granules. According to Neusch, bacteria are produced in the interior of animal or vegetable cells without any lesion and without coming from the air. To demonstrate this he plunges divers fruits under water, in saline or acid liquids (phosphates, sulphates, carbonate of potassa, etc.), and he finds there bacteria; but, according to him, these are not living organisms, properly so called, but abnormal cellular vegetations.

M. Servel, decapitating some guinea-pigs, caused the heads, the livers, and the kidneys to fall into a solution of chromic acid, 1 to 100. At the end of several days, the superficial parts were hardened; but the centre was softened, and filled with bacteria.

The presence of bacteria in eggs has several times been verified, and the heterogenists have hastened to draw an argument from this fact in favor of their theory. M. Gayon explains the appearance of these organisms in the eggs of birds by their presence in the normal state in the oviducts.

Finally, Bastian, having succeeded in obtaining bacteria in liquids which he believes deprived of every germ, believes in their spontaneous generation. The following is a *résumé* of his experiment: Normal acid urine is brought to the boiling-point, then a solution of potash (in sufficient quantity to neutralize the volume of urine employed) is also brought to the boiling-point; after cooling, the two liquids are mixed, and the whole placed in an oven at 50°. At the end of two or three days, bacteria are developed.

Pasteur points out three causes of error in the experiment of Bastian: 1. The germs may come from the urine; 2. The germs may come from the solution of potash; 3. The germs may be furnished by the vessels employed in the experiment. In support of this criticism, Pasteur has made some similar experiments, guarding against these causes of error, and has not obtained bacteria.

DISSEMINATION OF BACTERIA IN AIR AND WATER.

Air. — The experiment of Pasteur for gathering atmospheric germs is well known. He fixes a glass tube in an aperture made in a window-blind. The extremity of the tube, which communicates with the open air, is closed with a plug of cotton, to the other extremity is attached an aspirator. When the air has filtered through the cotton for some hours, this is examined, and is found to be filled with germs.

Before Pasteur, Ehrenberg and G. de Claubry had already announced the presence in the air of the eggs of infusoria. Robin had also recognized that the atmosphere contains, in addition to all sorts of débris, spores, pollen-grains, portions of insects, and rarely the eggs of infusoria. More recently Maddox and Cunningham, by the aid of an aeroscope invented by the former, gathered numerous microbes, as well as *bacteroid particles.* Tyndall, by causing a ray of light to enter a darkened chamber, has rendered visible all these minute corpuscles. His researches show that the optical examination of air enables us to determine in an exact manner the presence or absence of germs.

Let us also mention the experiments recently made by Miquel in the park of Montsouris. This observer has found in the atmosphere a considerable number of germs. For the forms of which the diameter exceeds 2 μ, he has ascertained that "the average number of microbes in the air is feeble in winter and augments rapidly in spring, etc.; 2. That rain always diminishes the number of these microbes; 3. That rain-water introduced with the greatest precautions, into flasks with slender curved necks, first heated to destroy germs, rarely contains rotifers, etc., but always contains bacteria."

En résumé, the existence of germs can be demonstrated, 1, by direct research; and 2, by cultivation. Direct research may be made by the optical examination of the air (method of Tyndall), the microscopic examination of dust (method followed by Marié-Davy, Tissandier), the examination of particles obtained by filtration, by gathering germs with an aeroscope, by condensation of atmospheric moisture upon refrigerating vases, etc. The cultivations consist in exposing to the air which is to be examined some liquids in which all pre-existing germs have been destroyed (Pasteur, Tyndall, etc.). This method has shown that liquids exposed in an atmosphere deprived of all germs does not undergo putrefaction, but this occurs as soon as the access of air not deprived of germs is permitted (Tyndall).

All of these methods give concordant results; deposits containing germs of various kinds are always obtained. But this objection presents itself to the mind: Do the bacteria obtained by cultivation exist in the atmosphere? or do they come from germs which have developed rapidly upon finding a favorable medium? From the experiments of Cohn, Miquel, etc., it is known that the atmosphere contains very few adult bacteria. Miquel in a recent communication says, in effect, that bacteria are rarely found in the air in a complete state, but rather under the form of shining points, difficult to distinguish directly one species from another. Are not these brilliant points *Micrococci?* In other terms, the air contains permanent spores, organisms which, as we shall see in speaking of the reproduction of the bacteria, develop at a certain period of the existence of the adult forms, in their interior, which escape from the sporogenous filament, are drawn into the air by the evaporation of the liquid containing them, or, after dessication, by the winds. These spores are the point of departure of epidemic foci, and their extreme lightness explains how readily they are disseminated by the winds.

Water. — Water contains considerable quantities of bacteria and especially of germs. Their presence in atmospheric water is established by the experiments of Lemaire and Gratiolet, — and after them by more recent observers, — by means of condensers filled with ice, and placed in the fields and for comparison in closed apartments. Rindfleisch has since expressed the opinion that the vapor of water does not contain spores or bacteria, and that telluric waters alone contain them; but Billroth, Cohn, and others have proved that Rindfleisch was too positive in his statement.

It is not surprising that telluric waters contain such a quantity of bacteria that their existence is admitted by all. The dust gathered upon the surface of stones, of leaves, of fruits, etc., shows upon microscopic examination an abundance of germs (Marié-Davy, Tissander); the washing of these objects and of the soil by the rain transports them into the rivers and from the rivers to the sea, which contains considerable quantities of them.

Thus, a drop of water from the Seine, according to Pasteur and Joubert, is always fecund, and may give birth to several species of bacteria. The distilled water of laboratories also contains germs, and these of so small a diameter that they pass through all filters.[1] Cohn has proved that some are not arrested by a super position of sixteen filters. The only waters which do not contain them are those drawn from the very source of a spring.

Dissemination of Bacteria in the Human Organism.

If bacteria are so generally disseminated in the great external media, it is not surprising that they are found on the surface of the human body and in the interior of the organs in communication with the exterior. But to account for their presence in the interior of organs we find ourselves in presence of two hypotheses: one admitting the spontaneous production of these organisms in the interior of the tissues, the second explaining it by the introduction through the membranes of the germs of bacteria from without.

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[1] Having been directed by the National Board of Health to make some experiments with a view to confirming or disproving the results of Klebs and Crudelli, who claim to have found the germ of malarial fevers in the atmosphere of the Pontine marshes near Rome (their *Bacillus malariæ*), I aspirated ten gallons of air on the edge of a swamp in the vicinity of New Orleans, through 4 c.c. of distilled water. Upon examining this water with the microscope on the following morning, I was surprised to find a large number of actively moving bacteria and monads (*Monas lens*). To make sure that these really came from the air, I examined my distilled water, which had been standing in the laboratory for several weeks (in a bottle, corked, but occasionally opened as distilled water was required) and found the same forms present in considerable numbers, not so numerous, however, as in the water through which swamp air had been drawn. As the germs were present in the distilled water, I presume that the passing of air through it for several hours, and the organic matter contained in it, favored the development and multiplication of these micro-organisms. Subsequent experiments with freshly distilled water gave very different results as to the number of organisms found. **See fig. 2, plate vii.—G. M. S.**

In truth, the cutaneous surfaces are penetrated with difficulty by germs, although the hairs upon the surface of the body serve to collect them. The short hairs in the nares prevent, to some extent, the atmospheric germs from penetrating into the bronchi, but this protection is not sufficient; and, notwithstanding the mucus of the nasal fossæ and of the pharynx, they may be found in the alveoli of the lungs, if we may believe Rindfleisch and Eberth. Do the bacteria pass into the blood? They may be transported in food and drink into the alimentary canal, where an elevated temperature, the presence of saliva, etc., favor their development. On the other hand, the acid secretions of the stomach, the bile, and the pancreatic juice moderate, if they do not prevent, the multiplication of these organisms.

The presence of bacteria in normal blood and urine, or their occasional entrance into these fluids, are important questions, which have induced many contradictory researches, but which are not yet definitely settled.[1]

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[1] "If there is any organism in the blood of yellow fever demonstrable by the highest powers of the microscope as at present perfected, the photo-micrographs taken in Havana should show it. *No such organism is shown in any preparation photographed immediately after collection.* But in certain specimens kept under observation in culture cells, hyphomycetous fungi and spherical bacteria made their appearance after an interval of from one to seven days. The appearance of these organisms was, however, exceptional; and in several specimens taken from the same individual at the same time, it occurred that in one or two a certain fungus made its appearance, and in others it did not. This fact shows that the method employed cannot be depended upon for the exclusion of atmospheric germs, but does not affect the value of the result in the considerable number of instances in which no development of organisms occurred

Two kinds of researches have been undertaken for the purpose of discovering germs in normal blood. The direct method, or microscopic examination, has given results very much disputed. The blood contains, indeed, a considerable number of little granules, of which the nature is doubtful, and which it is difficult to distinguish from *Micrococcus.* Thus, while Lüders asserts that normal blood contains germs, or spores, which only await a favorable alteration in the fluid in order to develop themselves, Rindfleisch formally denies their existence.

The indirect method, which consists in cultivat-

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in culture cells in which blood, in a moist state was kept under daily observation for a week or more.

"The method employed seemed the only one practicable for obtaining blood from a large number of individuals without inflicting unwarrantable pain and disturbance upon the sick. It was as follows: One of the patient's fingers was carefully washed with a wet towel (wet sometimes with alcohol and at others with water), and a puncture was made just back of the matrix of the nail with a small triangular-pointed trocar from hypodermic syringe case. As quickly as possible a number of thin glass covers were applied to the drop of blood which flowed. And these were then inverted over shallow cells in clean glass slips, being attached usually by a circle of white zinc cement. In dry preparations, which are most suitable for photography, the small drop of blood was spread upon the thin glass cover by means of the end of a glass slip.

"The thin glass covers were taken from a bottle of alcohol, and cleaned immediately before using; and usually the glass slips were heated shortly before applying the covers, for the purpose of destroying any atmospheric germs which might have lodged upon them. These precautions were not, however, sufficient to prevent the inoculation of certain specimens by germs floating in the atmosphere (*Penicillium* and *micrococci*); and in nearly every specimen the presence of epithelial cells, and occasionally a fibre of cotton or linen, gave evidence that under the circumstances such contamination was unavoidable. It is therefore believed that any organism developing in the blood of yellow-fever, or of other diseases collected by the method described, or by any similar method, can have no great significance, unless it is found to develop as a rule (not occasionally) in the blood of patients suffering from the dising normal blood in flasks perfectly closed, has also given some favorable results, such as those of Hensen, Tiegel, Billroth, and Nedvedsky, and some unfavorable results, as those of Lüders and Pasteur. According to Nedvedsky, the blood "contains germs capable of undergoing in it, under certain circumstances, an ulterior development: these are the *Hémococcos*." If these germs do not give birth, normally, to bacteria, it is because the blood is as injurious to them as the most advanced stages of putrefaction (Billroth). If this hypothesis is true, it explains several embarrassing facts, such as the existence of *micrococci* in the pus of

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ease in question, and is proved by comparative tests not to develop in the blood of healthy individuals, obtained at the same time and by the same method.

"Tried by this test, it must be admitted that certain fungi and groups of micrococci, shown in photographs taken from specimens of yellow-fever blood collected at the military hospital and preserved in culture cells, cannot reasonably be supposed to be peculiar to or to have any causal relation to this disease." — *Preliminary Report of Havana Commission to National Board of Health.*

In subsequent observations upon the blood of malarial fever, of syphilis, and of leprosy, I have sometimes obtained a development of micrococci in culture cells where all possible precautions as to the exclusion of atmospheric germs had been taken, and in one case have seen the development of *Penicillium* in another of *Sarcina*. The last observation is, so far as I know unique, and I have still in my possession the culture-slide containing numerous masses of *Sarcina*, presenting the characteristic arrangement of the cells in fours. This slide was put up at the bedside of a patient suffering from intermittent fever in the Charity Hospital, New Orleans. Every precaution was taken to exclude atmospheric germs. The patient's finger was washed with absolute alcohol just before making the puncture from which the little drop of blood was obtained. The question as to whether in this and similar cases the germs of the organism which develops come from the atmosphere or pre-existed in the blood is one to which I propose to give special attention; and, after further experiment, I shall discuss it in my report to the National Board of Health. — G. M. S.

closed abcesses, in cysts, in urine drawn from the bladder, etc.

§ 2. — NUTRITION AND RESPIRATION OF THE BACTERIA.

The bacteria, being organisms composed of a cell membrane of cellulose, and of protoplasmic contents, deprived of chlorophyll, must receive nutriment and respire in the same manner as all the colorless vegetables and all the inferior animals deprived of special apparatus, — that is to say, by endosmotic absorption.

Although the media in which the bacteria develop are various, yet, from the point of view of the nutritive function, they act everywhere according to the same laws. No matter in what medium they live, they must have water, nitrogen, carbon, and oxygen, as well as certain mineral salts which enter, but in quantities exceedingly minute, into the chemical constitution of all organized bodies.

Water. — This element is indispensable to the active life and development of the bacteria. Dessication arrests completely the movements of those which are mobile, and the functions of all the bacteria in general; but it does not kill them, at least if it be not prolonged beyond a certain time. The *Micrococci* of different kinds of *virus* are examples of the continued vitality of these organisms after dessication for a considerable time.

The bacteria present in this respect numerous variations according to the species and the period of development which they have attained. In the state of permanent spores, they are extremely tenacious of vitality. They resist for a long time not only dessication, but a considerable elevation of temperature.

Among the bacteria, some are developed in liquids, — the greater number, — others upon damp surfaces. The former can live in fresh water, sea-water, thermal waters, and the liquids of animal or vegetable organisms, etc. A surprising fact is, that the composition, so different, of fresh and sea water appears to have no influence upon the bacteria. We find in both all the species, from *Bacterium termo* to *Spirillum volutans*.

Nitrogen. — Pasteur has demonstrated that it is not necessary that the nitrogen which is to serve as nutriment to the bacteria should be in the form of albumen, but that these organisms can take possession of it in the form of ammonia.

In fact, in *Pasteur's solution*, composed as follows: —

Distilled water	100.
Sugar candy	10.
Tartrate of ammonia	1.
Ashes of one gramme of yeast .	0.075.

the bacteria increase sometimes with such rapidity that they interfere with the development of the alcoholic ferment.

Cohn, in order to better observe the phenomena and to get rid of the moulds, which the cane-sugar caused to develop too rapidly, employed the following culture-fluid:—

Distilled water	100.
Tartrate of ammonia . .	1.
Ashes of yeast	1.

Bacteria develop in this fluid wonderfully, which proves that sugar is not indispensable to them.

One other solution often employed is that of Mayer. It has the advantage of not requiring the employment of ashes of yeast:—

Phosphate of potash . . .	0.1	gramme.
Sulphate of magnesia . . .	0.1	,,
Tribasic phosphate of lime .	0.1	,,
Distilled water	20 c.c.	

Cohn adds to this 0.2 gr. tartrate of ammonia.

En résumé, the bacteria can take nitrogen, which they need in order to form their protoplasm, either from albuminous compounds, which they decompose, as in putrefaction, or in the form of ammonia, or, perhaps, by borrowing it from nitric acid, but this last source is not well established (Cohn).

Carbon.—In addition to the sources common to other organisms, the bacteria can take this important element of their composition, under certain circumstances, from the organic acids. Thus, when we cultivate bacteria in a solution containing only tartrate of ammonia with a small quantity of mineral salts (phosphoric acid, potash, sulphuric acid, lime, and magnesia), they develop rapidly, taking their carbon from the tartaric acid.

Cohn has endeavored to ascertain if other organic acids could be assimilated by the bacteria. By making use of succinate of ammonia, or neutral acetate of ammonia, he has been able to cultivate these microphytes. Besides, as Pasteur had already experimented with solutions containing lactates, and in which bacteria had developed until the salt had completely disappeared, we may admit that the bacteria can assimilate the organic acids, — tartaric, succinic, acetic, and lactic; but tartaric acid seems to furnish the best alimentary solution.

Other substances containing carbon are also assimilated by the bacteria, — cane-sugar, milk-sugar, glycerine, and even cellulose (according to Mitscherlich).

Cohn concludes, "that the bacteria multiply quite normally, and in great quantity, whenever they find the elements in solution which constitute ashes, and that they can take the carbon which they need from any organic substance containing it, and their nitrogen from ammonia, urea, and probably from nitric acid. The bacteria, then, resemble green plants, in that they assimilate nitrogen contained in their cells by taking it from ammonia compounds, which animals cannot do. They differ from green plants in that they cannot draw their carbon from carbonic acid, and only assimilate organic substances containing carbon, above all the hydrates of carbon and their derivatives; and in this respect they resemble animals."

Absorption. — How are these various substances absorbed? The observations of Grimm, Hoffmann, de Seynes, etc., permit us to assure ourselves that these organisms absorb by endosmosis the substances upon which they are nourished.

Grimm, upon examining with the microscope some particles of lemon containing bacteria and spores of algæ, saw a certain number of the former gather around a spore, and fix themselves to it by one of their extremities. They did not penetrate it; but when they abandoned it, the spore had diminished in volume, and lost a portion of its contents, while the bacteria had taken a greenish color.

Hoffmann has seen that these little organisms, when placed in a solution of carmine or of fuschine, after a time are colored an intense red, while the mucus surrounding them remains colorless.

De Seynes, also, from his observations upon the *vibrios* which accompanied some colored filaments of *Penicillium glaucum,* believes that bacteria are susceptible of absorbing coloring matters from vegetables and from animals with which they are in contact.

Oxygen. — The *rôle* of oxygen in the life of the bacteria has given rise to numerous controversies.

First, it seems *a priori* that the bacteria ought to act like all other living beings, and to respire like the other inferior organisms deprived of chlorophyll — that is to say by absorbing oxygen and eliminating carbonic acid. This is, indeed, the opinion of a great number of botanists. But, according to Pasteur, it is not so with the bacteria. When we examine what occurs in putrefaction, we find that at first certain species are developed (*Monas crepusculum*, *Bacterium termo*, etc.), which absorb all the oxygen dissolved in the liquid, and come to the surface where they form a thick veil; after this, other species of *vibrioniens* appear, which are developed in a medium entirely deprived of free oxygen, by borrowing this gas from the fermentable matters contained in the liquid. These chemical decompositions constitute putrefaction.

The first of these organisms, regarding the nature of which Pasteur has long been uncertain, are *aérobies:* they live in contact with the air, and have need of oxygen. The second, *anaérobies*, not only have no need of oxygen, but are killed by it.

These differences in the respiration of organisms belonging to the same group are not admitted by a great number of recent observers. Hoffmann, among others, says expressly: "These little beings cannot live without air, I should say without oxygen: if this gas is wanting, they cease to move and do not multiply at all. If a drop of liquid full of bacteria is placed upon a glass slip, then covered by a piece of thin glass, the active bacteria will all approach gradually to the margins of the cover; and it is there that at the end of several days, after the successive death of the greater number, some are still found endowed with life and movement. If a similar preparation is at the same time protected by an impermeable cement against dessication and against the introduction of atmospheric air, all movement among the bacteria will cease at the end of two minutes, provided, however, that no air bubble has been imprisoned with the liquid."

The influence of oxygen upon the life and development of bacteria is also very manifest in an experiment recently made, and not yet published, by Toussaint, who has been kind enough to communicate it to me.

In studying the development of the spores of *Bacillus anthracis* in the moist chamber of Ranvier, Toussaint has observed the following curious facts, which offer a striking analogy to those above mentioned, borrowed from Hoffmann. "The bacteria, which occupy the central portion of the moist chamber and which by reason of their situation receive very little oxygen from the groove, are soon arrested in their development; while those which occupy the borders are long and heaped up in immense numbers, those in the centre remain small, formed of two, four, or five articles, which are easily separated from each other; they soon cease to grow and are not transformed into spores."

Cohn is also as explicit. "There is no doubt," he says, "that the complete development of *Bacillus*, and above all reproduction by means of spores, is only made under the influence of free access of air."

We might explain the contradictory facts of Pasteur by admitting, with Cohn, that the appearance of different *rôles* played by the *aérobies* (*Bacterium*) and the *anaérobies* (*Bacillus*) is simply due to a veritable struggle for existence which takes place between the *microbacteria* and the *desmobacteria*.

ACTION OF VARIOUS AGENTS UPON THE BACTERIA.

In this paragraph I shall pass in review the action of temperature, of movement, and of various antiseptics.

Temperature.—It is very important to study the manner in which bacteria comport themselves under extreme variations of temperature. It is, indeed, upon the results furnished by these researches that a great part of the arguments opposed to the panspermatists by the heterogenists are based.

We shall consider the influence upon bacteria of moderate temperatures and of extremes above and below zero.

Moderate temperatures—that is to say those which are comprised between 25 and 40° (77 to 104° Fah.)—are generally favorable. The most favorable has been found to be 35° (95° Fah.) (Onimus).

The degree of resistance to extreme temperatures is very variable, according to the species. Thus, according to Frisch, a temperature of 45 to 50° (113 to 122° Fah.) is sufficient to kill *B. termo*, whilst 80° (176° Fah.) does not kill the "*Bactéridies*" (*Bacillus*).

The permanent spores are especially remarkable by the tolerance which they possess for high temperatures. They have been subjected to 100° (212° Fah.) (Schwann), 110° (Pasteur) and even 130° (Schrader) without losing their power of germinating.

We must, however, recognize that the results of the experimenters offer the greatest diversity, the result, according to Cohn, of the difficulty of obtaining an equable distribution of the heat in the media, which are generally bad conductors.

Cohn has arrived at the following conclusions as the result of numerous experiments made upon the *Bacillus* of hay infusions: —

1. At a temperature of 45 to 50° (113 to 122° Fah.) the *Bacillus* still multiplies rapidly, and forms as usual membranes and spores, while the other schizophytes existing in the infusion of hay are at this temperature incapable of multiplication.

2. At a temperature of 50 to 55° (122 to 131° Fah.) all reproduction and development of *Bacillus* ceases. It neither forms pellicles or spores; the filaments are killed, the spores, on the contrary, preserve, for a longer time (for at least seventeen hours) the property of germinating.

3. While infusions of hay are generally sterilized by a temperature of 60° (140° Fah.) or more, prolonged during twenty-four hours, certain spores of *Bacillus* seem able to endure a temperature of 70 to 80° (158 to 176° Fah.) during three or four days without losing the power of germinating.

By some experiments made with refrigerating mixtures, Cohn has ascertained that the bacteria are not killed by very low temperatures, acting even during several hours, — 18° for example (0° Fah.). But they are benumbed at a temperature of 0° (32° Fah.) and probably at a temperature a little higher, losing the power of movement and of reproduction, and consequently their action as ferments. They preserve, however, their capacity to resume their activity at a more elevated temperature.

Frisch has pushed the experiment still further than Cohn. By the evaporation of carbonic acid, he has produced as low a temperature as — 87° (— 123° Fah.) in liquids containing bacteria, without destroying the vitality of these organisms, development having subsequently occurred of *coccos* and of bacteria. Congelation, then, cannot serve to destroy the organized ferments.

Let us add, however, that if the passage to extreme temperatures is too sudden, there is then an alteration (destruction ?) of these organisms (Schumacher).

Movement. — We would not have consecrated a paragraph to the action of movement upon bacteria, if Crova had not recently asserted that movements impressed upon a liquid containing bacteria completely arrests their development. This is an assertion in complete opposition to all that we know of the physiology of these organisms, and which it is difficult to reconcile with the fact that bacteria may develop even in the torrent of the circulation.

Compressed Air. — We have just seen the influence of air, and especially of oxygen, upon the bacteria. When this agent is in a certain state of tension, it acts in a different manner. M. Paul Bert has proved that under a tension of twenty-three to twenty-four atmospheres all the putrefactive processes depending upon the development of vibrios cease to occur. Since, the same *savant* has found that the anatomical elements and even the red blood globules are killed by oxygen. These researches agree well enough with those of Grossmann and Mayerhauser upon the life of bacteria in gas. From their numerous experiments it appears that, under the influence of oxygen, there is an exaggeration of the activity of the bacteria; but if the oxygen is under a pressure of five to seven atmospheres, the bacteria live from six to twenty hours, then die, and cannot be resuscitated by atmospheric air.

Ozone causes a definite and almost instantaneous arrest of movement.

Other gases studied by the same *savants* have given the following results: —

Hydrogen at first causes an acceleration of movement, which is maintained for several days; then movement becomes less active, and finally it ceases altogether.

Carbonic Acid. — Contrary to the facts stated by Pasteur, this agent was found to paralyze the bacteria, and reduced them to complete immobility. If the carbonic acid is displaced by oxygen, the bacteria resume their activity.

Chloroform. — This substance, according to the researches of Müntz, arrests the vital phenomena of organized ferments. Müntz uses this character in order to recognize the soluble ferments, upon which it has no action.

Boracic Acid. — Since the labors of Dumas, which have demonstrated that boracic acid kills the inferior organisms by depriving them of their oxygen, this substance has been employed in various circumstances as an antiseptic.

Sulphate of Quinine. — The action of quinine, either in the state of chlorhydrate or of sulphate, is not yet well established. The experiments of Binz, Manasseïn, Krœvitsch, Bochefontaine, etc., have, in truth, given contradictory results.

Carbolic Acid. — The experiments of Manasseïn have demonstrated that $\frac{1}{20}$th per cent of carbolic acid is sufficient to prevent all development of living beings. It is employed with success in anthrax, in the treatment of wounds, etc.

§ 3.—REPRODUCTION OF THE BACTERIA.

It is well established that the bacteria can multiply by fission, and reproduce themselves also by the formation of endogenous spores.

Fission.—The multiplication by fission consists in a transverse division of the cell. When a bacterium has attained nearly double its ordinary length, we see, in the larger species, that the protoplasm becomes clearer in the central portion, and a partition forms in the median line separating the contained protoplasm into two portions. The partition, at first very delicate, becomes thicker, divides into two, and the two articles separate.

This phenomenon is produced more or less quickly according to the nature of the medium, its richness in nutritive material, the temperature, etc. When the growth is rapid, the new cells form more quickly than they separate, and are arranged in chaplets. Very often we only find them in this form, in strings of two to four cells coupled together. In some forms the transverse division is preceded by constriction near the middle of the cell. Before the two new cells are separated, the bacterium in this case presents the appearance of a figure 8, and seems to be a simple cell swollen at the two extremities.

Under other circumstances, and probably in consequence of a mucus transformation of the walls of the mother cells, the new bacteria are enveloped by a mass of glutinous substance. We have described these masses under the name of *Zoogl&oelig;a*.

The conditions which favor multiplication by fission are, a certain degree of temperature and a sufficient quantity of nutritive material. The higher the temperature, the more rapid is the segmentation of the bacteria, the more rapid their multiplication, — that is to say, up to a certain limit, variable with the species and beyond which the bacteria are destroyed.

The multiplication decreases when the temperature is lower, and ceases entirely in the vicinity of 0° (32° Fah.).

The influence of richness of nutriment is well seen in artificial cultivation. So long as the bacteria find the necessary aliment, in sufficient quantity, to form new protoplasm, they multiply with activity; but as soon as the organic matter is devoured, they cease to divide, fall to the bottom of the vessel, where they accumulate, motionless, and form a deposit more or less abundant.

The multiplication of the bacteria by binary fission has for result, if nothing occurs to interfere with the most favorable conditions, the invasion of the medium by an incredible number of these little beings, of which we can only form an idea by calculation.

" Let us suppose," says Cohn, " that a bacterium divides into two in the space of an hour, then in four at the end of a second hour, then in eight at the end of three hours, in twenty-four hours the number will already amount to more than sixteen millions and a half (16,777,220); at the end of two days this bacterium will have multiplied to the incredible number of 281,500,000,000; at the end of three days it will have furnished forty-seven trillions; at the end of about a week, a number which can only be represented by fifty-one figures.

"In order to render these numbers more comprehensible, let us seek the volume and the weight which may result from the multiplication of a single bacterium. The individuals of the most common species of rod-bacteria present the form of a short cylinder having a diameter of a thousandth of a millimeter, and in the vicinity of one five hundredth of a millimetre in length. Let us represent to ourselves a cubic measure of a millimetre. This measure would contain, according to what we have just said, 633,000,000 of rod-bacteria without leaving any empty space. Now, at the end of twenty-four hours the bacteria coming from a single rod would occupy the fortieth part of a cubic millimeter; but at the end of the following day they would fill a space equal to 442,570 of these cubes, or about a half a litre. Let us admit that the space occupied by the sea is equal to two-thirds of the terrestrial surface, and that its mean depth is a mile, the capacity of the ocean will be 928,000,000 of cubic miles. The multiplication being continued with the same conditions, the bacteria issuing from a single germ would fill the ocean in five days."

Reproduction by Spores.—The multiplication by fission, known to the earliest microscopists, has been until recently the only mode of propagation admitted by the authors. Thus M. de Lanessan, in the excellent article which he has devoted to the bacteria, says that the marvellous resources of modern science have not yet permitted us to recognize any other mode of propagation for these organisms.

However, M. Ch. Robin had already, in 1853, indicated the presence in *Leptothrix buccalis* of little round bodies, "which are perhaps spores." Pasteur has since, in 1865, recognized that "the vibrios of putrefaction and of butyric fermentation present a sort of ovule, or ovoid corpuscle, which refracts light strongly, either in the extremity or in the body of the articles." Later, the same *savant*, more explicitly, says clearly that these organisms have two modes of reproduction,—by fission and by interior spores ("*noyaux*").

Towards the same epoch, Hoffmann also pointed out a reproduction by free cellular formation in some bacteria. But we must come to the labors of Cohn, Billroth, and Koch, in order to find precise observations in this regard.

The formation of spores has been observed in *Bacillus subtilis* by Cohn, *Bacillus anthracis* by Koch, and in *Bacillus Amylobacter* by Van Tieghem.

PLATE VIII.

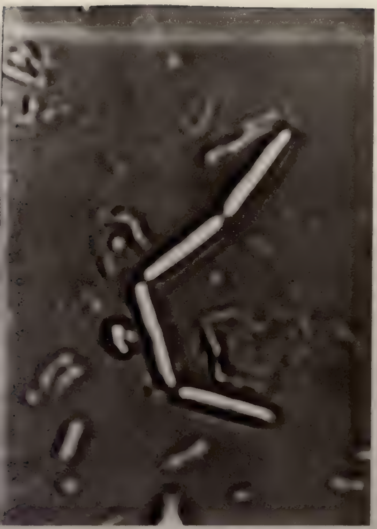

FIG. 1.

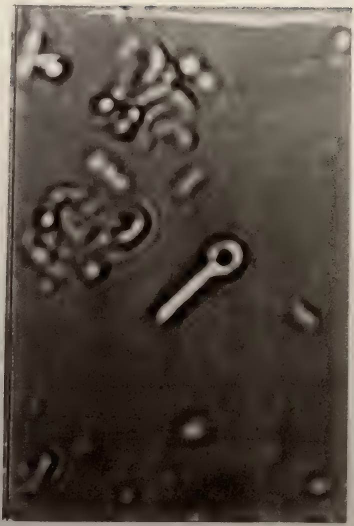

FIG. 2.

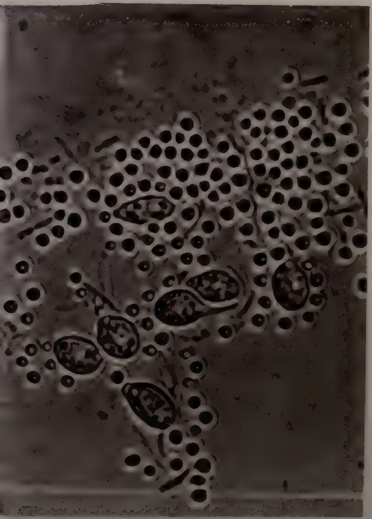

FIG. 3.

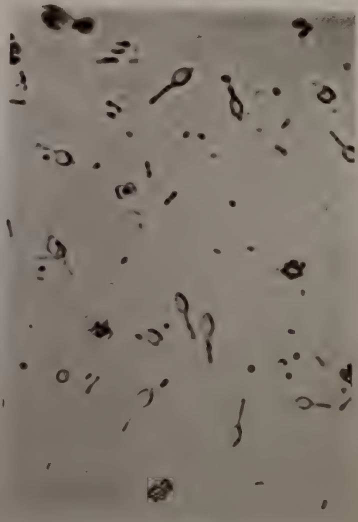

FIG. 4.

PLATE VIII.

FORMATION OF SPORES IN BACILLUS.

From photo-micrographs made in Havana and in New Orleans. Reproduced by permission of the National Board of Health.

FIGS. 1 and 2. — *Bacillus* (*ulna ?*) found in blood of yellow-fever patient (*post mortem*) five days after collection. × 3,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIG. 1. — Rods joined in leptothrix chain.

FIG. 2. — A single rod showing spore at one extremity.

FIG. 3. — Spores of Bacillus developed in rotten potato, New Orleans, April, 1880. × 1,500 by Zeiss's $\frac{1}{18}$ in. objective. The large cells are some species of Saccharomycete, which was also present in the same specimen.

FIG. 4. — Development of bacilli from spores, from culture experiment with fish gelatine solution. × 1,500 diameters by Zeiss's $\frac{1}{12}$ in. objective.

Cohn, who had in his first publications refused to the bacteria the property of reproduction by spores, thinking that the facts observed by Hoffmann related to different beings, has verified the experiments of Koch upon the development of *B. anthracis*, and has himself demonstrated similar phenomena in *B. subtilis*.

In culture experiments made with infusion of hay, we see, at a certain moment, in the homogeneous filaments of the *Bacilli* very refractive corpuscles making their appearance. Each of them becomes a spore, oblong or in the form of a short filament, highly refractive, and with well-defined outlines. We find the spores arranged in a simple series in the filaments. As soon as the formation of spores has terminated, the filaments can generally no longer be distinguished, and one would say that the spores were completely free in the mucus; but their linear arrangement shows always that they are produced in the interior of filaments. Little by little these dissolve, being reduced to a fine powder; and the spores fall to the bottom of the liquid, where they are found in abundance. The germination of the spore does not seem to occur in the same medium; but if we take a spore from the deposit formed in an infusion of boiled hay, and transport it into a new infusion, we see the spore swell up, and a short tube form itself at one of its extremities: at this moment it resembles a bacterium with a head. Soon the very refractive body disappears, the tube stretches out into a short rod of *Bacillus*, commences to move, and becomes jointed by transverse division.

Koch, in cultivating the bacteria of charbon in aqueous humor from the eye of the ox, has observed some facts exactly similar, both as to production of spores in linear series in the filaments of *Bacillus anthracis* and as to the germination of the spore and the birth of a new rod.

According to Van Tieghem, the development of *Amylobacter* is as follows: "The development of a *Bacillus* includes four successive periods. In the first, the body, cylindrical and slender, recently developed from a spore, stretches out rapidly, and is partitioned; the articles soon separate (*B. subtilis*), or remain united in long filaments (*B. anthracis*). This is the stage of growth and multiplication, two things which at bottom are but one.

"Secondly, the articles previously formed, having ceased to elongate and divide, increase sensibly in magnitude, becoming the seat of interior chemical transformations; and this increase in size operates according to circumstances, in three different manners, with some intermediate forms. Sometimes it occurs uniformly throughout the length of the article, which remains cylindrical; sometimes it is localized, either at one extremity, which is swollen like a tadpole, or in the middle of the article, which swells to a spindle shape. This is the stage of enlargement, or of nutrition, solitary and simultaneous, which prepares the following state.

"In the third period or phase of reproduction there is formed in each article so nourished a spherical or ovoid spore, homogeneous, highly refractive, having a dark outline. At the same time, the protoplasm which occupies the rest of the cavity disappears little by little, and is replaced by a hyaline liquid, which separates the spore from the membrane; this dissolves in its turn, and finally the spore is set at liberty. If the article is swollen in tadpole shape, it is in the terminal swelling that the spore has birth; if it is spindle-shaped, it is near the middle; if it is cylindrical, it may be at any point whatever, but is usually near one extremity. The spore when set free germinates under favorable circumstances. At a point where its circumference becomes pale, it gives out a little tube slightly more slender than itself, which elongates rapidly and divides. This fourth period of development or germinative phase brings us back to our point of departure."

Sporangia. — Finally, not only do the bacteria develop spores in the interior of their filaments, slightly modified in form, but we may also observe the formation of a veritable sporangium containing many spores. The unpublished observations of M. Touissant, Professor of Physiology in the Veterinary School of Toulouse, give this result, which he has kindly communicated to me.

In cultivating spores of the bacteria of charbon in the serum of the blood of the dog, under the microscope, in the warm chamber of Ranvier, Toussaint has seen the filaments take a transverse diameter almost double the ordinary diameter, then the protoplasm of the filament to gather together at certain points, — a fact clearly made out, as in the parts where the protoplasm was wanting the bacteria had lost all refractive power. Finally, at a later period the points occupied by the condensed protoplasm augment considerably in volume, and form some ovoid organs more or less elongated, or swollen into a ball, or in the form of a gourd at one extremity. In the interior of these sporangia, from three to six spores afterward form, clearly defined and highly refractive; then, finally, by breaking up of the membranous envelope the spores become free.

Toussaint has also followed in the same apparatus — moist and warm chamber of Ranvier — the mode of germination of the spores. The following are the most important facts: —

The spores are at first highly refractive and animated by brownien movements; at the end of half an hour to an hour, at a temperature of 37 to 40°, in urine, aqueous humor, or serum, the spores lose their refractive power, and their brownien movements cease almost entirely; then the spore assumes an aspect slightly granular, it becomes elongated in the direction of its greatest diameter (they are oval). After two hours of cultivation, the bacterium has two or three times the dimensions of the primitive spore; the elongation makes rapid progress, and four to six hours from the commencement of the cultivation, some may be found to occupy the entire field of the microscope. From this moment the phenomena which occur differ according to the conditions in which the bacteria are placed. Upon the edge of the air-groove in the moist chamber, the bacteria develop very rapidly, forming an interlaced mass; and in sixteen to eighteen hours, spores may be seen to appear in their interior, — above all, if the preparation has been exposed to light. Often, in this case, the transverse partitions of the filament cannot be seen. If, on the contrary, the bacterium has not been exposed to light, the spores are a longer time in showing themselves (ten or twelve hours more), and almost always division of the filament precedes their formation. In that case, a spore usually appears at each end of the segment in such a manner that the spores belonging to two successive segments are nearer to each other than those in the same segment. Often, also, a spore aborts in a segment (Toussaint).

We have seen above, in speaking of the respiration of bacteria, that the same observer has noted in the course of his experiments some phenomena proving the evident influence of oxygen upon the development of *Bacillus*. It is the same for the formation of spores. And upon this point Toussaint makes the very just remark that the phenomena occur in a different manner in culture experiments and in the human organism. In charbon, the bacteria never form spores. They remain always relatively short, even in the points where they form extra-vascular masses, and where consequently we cannot invoke the movements of the liquid in order to explain their division. The bacteria of charbon, then, take but little oxygen from the tissues: they do not vegetate luxuriantly in the organism; and certainly, if we judge by a calculation necessarily approximative, their development is seven or eight times less rapid than in the strongly oxygenated serum of culture experiments (Toussaint).

Polymorphism. — The spores of which we have traced the genesis constitute those germs of which the origin has for a long time been misunderstood, — those *permanent spores* or *durable spores* (Dauersporen), thus called because of their remarkable degree of resistance to temperature, desiccation, and all the agents which kill adult bacteria or arrest their development.

These "organs" are disseminated in great numbers in various media under the form of little rounded corpuscles absolutely similar to the *micrococci* from which it is absolutely impossible to differentiate them. It is, indeed, very probable that the greater part, if not all of these organisms, are the spores of filiform bacteria.

In the impossibility of recognizing these forms so nearly related, of referring them to such or such a determined organism, the attempt has been made to cultivate them, in order to follow their development. We have just seen the results of this cultivation for the *Bacillus;* but, in the hands of the greater number of experimenters, the results of such culture experiments are far from being so certain. Not having succeeded in removing them completely from the invasion of foreign germs, the greater number have seen the most diverse forms develop themselves, and from this have inferred the most remarkable transformations.

Thus, Hallier pretends to have observed the transformation of *Micrococcus* into various fungi, such as *Mucors*, *Ustilago*, etc. The M. of vaccinia comes from *Torula rufescens*, which is itself a phase of development of *Ustilago carbo;* the M. of human variola is derived from a fungus having sporangia and pycnidia, related to *Stemphylium sporidesmium;* that of the variola of animals from *Cladosporium* (*Pleospora*) *herbarum;* the M. of the blood of scarlatina belongs to the g. Tilletia; those of glanders and of syphilis from a *Coniothecium*, etc. In the same way Letzerich has referred the M. of diphtheria to another *Tilletia*, the *T. diphtherica*.

The transformation of bacteria into "*levûres*" (yeast fungi), and these into *Penicillium*, has been admitted by Hallier, Trécul, and others. But the researches of Brefeld and de Seynes have shown us that this is far from being demonstrated; indeed, in his numerous cultivations, de Seynes has never been able to verify such an affiliation; and Nägeli in his turn has never been able to obtain a transformation of schizomycetes into budding fungi.

It is the same as regards the transformation of bacteria into moulds and mildews. In some recent cultivations of moulds, made with care, Nägeli has never observed the formation of schizomycetes, and reciprocally. Are we not permitted to believe, now that we know of the formation of sporangia among the bacteria, that the microscopists who sustain a polymorphism so extended, have taken these organs, of which they have not been able to follow exactly the development, for the sporangia of *Mucorini?* This explanation is the more admissible as Trécul has seen the bacteria "swell up, and transform themselves separately," a phenomenon quite identical to that observed by Toussaint.

En résumé. The only change of form well demonstrated in the present state of science, and the only one which can be compared to natural polymorphism, such as it exists in a great number of fungi, consists in the transformation of spores into *Bacteria*, *Bacteridia*, *Vibrios*, etc., and in the different modes of grouping that the cells of bacteria take in becoming *zooglœa*, *mycoderma*, *leptothrix*, etc. To go further would be to lack prudence and scientific criticism.

CHAPTER II.

DEVELOPMENT OF THE BACTERIA IN DIFFERENT MEDIA.

In studying the conditions of life and of development of bacteria in the different media, natural and artificial, in which they are met, we will consider the actions which they determine (or that they accompany) as particular cases of their nutrition and of their reproduction. We will constantly take, then, their normal physiology as our point of departure; and we will try to explain in this way the phenomena, so diverse, with which they are associated, — fermentations, putrefactions, contagion of infectious maladies, etc.

It is especially interesting to study the *rôle* of bacteria in *non-nitrogenized chemical media,* where they accompany the phenomena called fermentation, properly so called; in nitrogenized media, vegetable or animal, which they transform, as a result of special fermentations, which constitute putrefaction; in the human organism, where they accompany frequently, if not always, the development of certain affections having special characters. This will be the object of so many paragraphs.

§ 1.—Rôle of Bacteria in Fermentations.

We say that a liquid is fermenting whenever modifications occur in its chemical constitution, as a result of the nutrition of organized beings.

Two kinds of fermentation are commonly distinguished. In the first group (false fermentations) are arranged those which are produced by soluble quarternary substances (diastase, soluble ferments) secreted by living cells, from which they may be separated in order to study their action upon fermentable liquids. This action is comparable to that of certain mineral acids, which operate in the same manner, either by the breaking up of molecules with addition of water or by the phenomena of hydration. Veritable chemical reagents, when these substances are once precipitated from their solutions, purified and dried, they preserve their properties indefinitely. A sufficient elevation of temperature seems to destroy the edifice of their molecule; for they lose all their specific power after having been subjected to a temperature more or less elevated, but always inferior to 100° (212° Fah.).

In the second group (true fermentations) are joined all the phenomena of chemical modification which appear intimately united to the development of inferior organisms,—algæ or fungi (figured ferments). Compressed oxygen by killing these ferments, and chloroform by suspending their vital functions, arrest the progress of these fermentations, while the same agents do not modify at all the action of soluble ferments. According to Dumas, borax has, on the contrary, the property of entirely destroying the activity of soluble ferments without absolutely preventing certain true fermentations,—for example, the alcoholic fermentation of glucose. We will see further on that this property of borax has been utilized in the treatment of catarrh of the bladder and of certain virulent affections.

Although at first view these two groups of phenomena seem very different, they may, however, be compared the one with the other. Without speaking of the ammoniacal fermentation of urine, which, as we shall shortly see, may be arranged in either of these groups, we may admit that the only difference between these two series of chemical modifications consists in the fact that in one case the true fermentations being the last term in the interior nutrition of the cell have their seat in the interior of the cell itself; while in the other the first terms of nutrition are always extra-cellular phenomena, having for effect, as Cl. Bernard has shown, to render assimilable or diffusible in the interior of the organism the aliment necessary to the development of every organized being (transformation of starch into glucose, of sugar into glucose, emulsion of fats, liquefaction of albuminoid substances).

The study, from a chemical point of view, of these phenomena of nutrition, of these fermentations, since such is their name, has not yet made much progress, and it would be difficult to make a rational classification of them in the present state of our knowledge. I will not then seek to classify them, but will content myself with describing them successively, commencing with the best known. I shall only speak of the fermentations caused by the development of bacteria, leaving, consequently, the fermentation which has been best studied, — the alcoholic. I adopt the following order: —

1. Acetic fermentation of alcohol.
2. Ammoniacal fermentation of urine.
3. Lactic, viscous, and butyric fermentations of sugar.
4. Putrefaction, or nitrification.

Acetic fermentation. — The transformation of wine into vinegar is a phenomenon long known and utilized. From a chemical point of view, this transformation is due to oxydation of the alcohol. The following formula represents this reaction: —

$$C^2H^6O + O^2 = C^2H^4O^2 + H^2O.$$

The agent of this oxydation is a micro-organism called *Mycoderma aceti.* It belongs to the group of the *microbacteria,* and we have already given the botanical description of it (page 83); but its development presents some interesting peculiarities which we think it proper to indicate in the language of M. Duclaux: —

"These little beings reproduce themselves with such rapidity that by placing an imperceptible germ upon the surface of a liquid contained in a vat having a surface of one square metre, we may see it covered, in from twenty-four to forty-eight hours, with a uniform velvety veil. If we suppose that there are three thousand cells in a square millimetre, which is below the truth, this will give for the vat three hundred *milliards* of cells produced in a very short time."

"The *Mycodermi aceti* is not always the same. Usually it forms upon the surface of a liquid a soft-looking veil, smooth at first, then wrinkled, which is with difficulty submerged and moistened. If a glass rod is plunged into the liquid, it pierces this veil; and when it is withdrawn, a portion remains attached to the rod; and the opening made immediately disappears, being occupied by the veil which seems never to have room enough in which to extend itself. In some unpublished experiments I have frequently observed another form of veil, dryer, finer, and sometimes showing prismatic colors. This veil does not wrinkle, but is covered with crossed undulations, having sharp edges, which recall the surface of a honeycomb. Sowed upon the surface of various liquids, it reproduces itself identically, and it is difficult not to consider it a different form of the preceding. Finally, I have also met a species of *mycoderma* producing well-developed veils, but having scarcely any acetifying power, and reproducing itself with this character."

"It is difficult to distinguish these forms the one from the other, by the microscope, because of their minuteness. We may, however, say that the second which I have described is sensibly smaller than the first, and the third more attenuated than either of the others. However, the differences are feeble."

This veil is called the *mother of vinegar*. The liquid in which this mycoderma is cultivated should be a little acid, containing one-half per cent of acetic acid, for example. Under these conditions the *Mycoderma vini* (a species of Saccharomycete), the formation of which should be avoided, finds conditions unfavorable to its existence. Indeed, this second organism, commonly called *flowers of wine*, has an action quite different from that of the *Mycoderma aceti.* It consumes the alcohol entirely, transforming it into water and carbonic acid: it also consumes the acetic acid. We must sow the *M. aceti*, if we do not wish to see the *M. vini* develop in its place, as the germs of the latter seem more widely diffused in the air.

In order that the acetification may occur, the oxygen of the air is necessary. Once submerged, the *M. aceti* develops, but no longer produces acetic acid. It is even probable that it consumes the acetic acid already formed, reducing it to the state of water and carbonic acid. It is the same when, developing upon the surface, it has transformed all the alcohol. "In effect, it is not then arrested in its work; and without changing form or mode of action, it carries the oxygen of the air to the acetic acid which it has produced, transforming it into carbonic acid and water. If we add some alcohol to the liquid, the phenomena change: the acid is respected, and the alcohol is transformed anew into acetic acid" (Duclaux). According to the experiments of Mayer, the maximum of acetifying power is obtained between 20° and 30° (68° to 86° Fah.), and this power is lost below 10° (50° Fah.) and above 35° (95° Fah.).

Ammoniacal Fermentation of Urine. — When urine is freely exposed to the air, we perceive at the end of a short time that it has become strongly ammoniacal. The urea is transformed into carbonate of ammonia by the addition of water: —

$$CO(NH^2)^2 + H^2O = CO^2 + 2NH^3.$$

Müller suspected that the deposit of altered urine, of which Jacquemart had already recognized the particular activity, was an organized ferment, but this was only an induction drawn from the analogy with beer yeast. Pasteur showed that this sediment is formed of a mass of spherical globules, united in chaplets, which he considers the agent of ammoniacal fermentation. These globules are *Micrococcus ureæ,* Cohn, which we have already described (page 75).

This bacterium lives in the interior of the liquid, and not on the surface like the *Mycoderma aceti.* Acidity is an obstacle to its development; alkalinity, on the contrary, favors it within certain limits. Van Tieghem has even seen the fermentation continue until the liquid contained thirteen per cent of carbonate of ammonia.

What is the mechanism of this fermentation?

M. Musculus has shown that we may obtain from altered urine a soluble ferment upon adding to it highly-concentrated alcohol: a precipitate is formed, which may be filtered and dried. This precipitate, not at all organized, transforms urea into carbonate of ammonia. A temperature of 80° (176° Fah.) destroys it. This diastase appears, then, to be a secretion of the *Micrococcus ureæ;* and perhaps the *rôle* of the bacteria is limited, in the phenomena of fermentation, to the formation of this secretion alone. The ammoniacal transformation of urine would consequently enter into the group of fermentations by the varieties of diastase.

According to Arnold Hiller, if carbolic acid be added to urine, it does not become alkaline; on the contrary, the acidity is even augmented, and that notwithstanding a considerable number of bacteria which develop in it. Has the carbolic acid killed the *Micrococcus ureæ,* leaving the field free to other organisms capable of living in an acid medium, and of producing other transformations of the constituents of the urine? In the memoir which we here cite, the author, resuscitating the ancient opinion of Liebig, wishes to demonstrate that the decomposition of dead organic matters, and putrefaction in general, are phenomena purely chemical, — these decompositions being determined by the presence of organic substances, themselves undergoing transformations.

We will not stop to consider these views, long since refuted: the experiments upon which they are founded are easily criticised. It is sufficient for me to say that they are in formal opposition with all the observations contained in modern works upon this question.

It is especially in relation to ammoniacal fermentation that the question of spontaneous generation has been discussed. We have already seen the results arrived at, and will not return to this subject. Let us, however, mention before closing an interesting work by MM. Cazeneuve and Livon, in which are reported some experiments which prove that urine never ferments in a healthy bladder.

Lactic, Butyric, and Viscous Fermentations of Sugars. — Saccharine liquids, left to themselves, are susceptible of divers fermentations, which may occur separately or simultaneously. Those which have been best studied are three, — the lactic, the butyric, and the viscous fermentations. We will describe them successively.

1. *Lactic Fermentation.* — Under the probable influence of a bacterium (*ferment lactique* of Pasteur) glucose and the substances susceptible of furnishing it, such as mannite, malic acid, etc., are transformed into lactic acid.

From a chemical point of view, there is in this nothing more than a molecular change, lactic acid having the same composition as glucose.

Taken in mass, the lactic ferment resembles beer-yeast; its consistence is, however, a little more viscous, and its color more gray. But under the microscope, the aspect is very different, as we have seen in describing *Bacterium lineola.*

An interesting point concerning this fermentation is the action of acids upon the bacteria which produce it (presumably). As soon as the medium becomes acid, even by the lactic acid produced, the transformation is arrested. It resumes its course, if chalk or carbonate of soda is added to the liquid.

The most suitable temperature seems to be 35° (95° Fah.).

We know but little about this fermentation. "It merits, however, to be better studied. It is this which causes the spontaneous coagulation of milk: sugar of milk is transformed into lactic acid, which coagulates the caseine. We often see it occur in beef juice or in sour starch water; it must play a part in the formation of sour krout, and intervenes very certainly, and perhaps more than the alcoholic fermentation, in the preparation of bread. Finally, it very easily invades beer, which of our domestic drinks is most exposed, because of its slight acidity, to become the seat of this fermentation. All of these facts render it interesting, so much the more as it is rarely exempt from complication, and is frequently accompanied, for example, by a commencement of butyric fermentation, far more disagreeable in its products" (Duclaux).

2. *Butyric Fermentation.* — This is, in fact, always preceded by a lactic transformation, and it is by an ulterior modification that the lactic acid produces the butyric acid. The organism which accompanies it is a bacterium very nearly allied to *Bacillus subtilis*, Cohn.

The reaction represented by the phenomena, from a chemical point of view, is the following: —

$$\underbrace{2C^3H^6O^3}_{\text{lactic ac.}} = \underbrace{C^4H^8O^2}_{\text{butyric ac.}} + 2CO^2 + H^4.$$

According to Pasteur the butyric ferment belongs to his class of *anaérobies*.

This fermentation resembles putrefaction in a great many particulars. Indeed some authors include it under the same head.

3. *Viscous Fermentation.* — Wines often change so that they contain a mucilaginous substance and mannite. This viscous matter has the same composition as gum or dextrine ($C^6H^{10}O^5$); at the same time it disengages carbonic acid.

In the fermenting liquid, we find an organism which is not yet sufficiently studied. "There are chaplets of little spherical bodies, of which the diameter varies sensibly, according to the kind of wine attacked by this malady (Pasteur).

Pasteur has proposed the following formula: —

$$25(C^{12}H^{22}O^{11}) + 25H^2O = 12\underset{\text{gum.}}{(C^{12}H^{20}O^{10})} +$$

$$24\underset{\text{mannite.}}{(C^6H^{14}O^6)} + 12CO^2 + 12H^2O.$$

which represents the phenomena well enough as it usually occurs. There is produced in the vicinity of 51.09 of mannite and 45.5 of gum for one hundred parts of sugar. But sometimes the gum exceeds the mannite in quantity. In this case, according to Pasteur, we can always verify in the liquid the presence of a larger ferment of a different nature; and the same author adds that, perhaps, in this case the increased production of gum results from the presence of this second ferment, which transforms the sugar only into gum, without any correlative formation of mannite. But this ferment has never been isolated. M. Monoyer has explained the variation in the proportion of gum in another manner (see his thesis for the doctorate in medicine, Strasburg, 1862).

White wines are more subject than red wines to this fermentation, called *graisse des vins.* According to M. François, the absence of tannin in the white wines is the cause of this disease, and it may be prevented by adding this substance. This remedy is even very highly appreciated in champagne, according to Pasteur. What is the exact action of the tannin upon the gummy ferment? The only means of knowing is by cultivating this ferment in a state of purity and treating it with this agent.

We have united together the lactic, butyric, and viscous ferments, because all three manifest themselves in the same liquids,—wines, beer, sweetened water, etc.; and because they have for effect the transformation of glucose. We ought to say a word here of some other inferior organisms, perhaps bacteria, observed also in the same liquids, but which have not been as well studied. Not only are they not known systematically, but we do not know precisely what is their chemical action upon the elements of the medium which nourishes them. I shall only enumerate them.

1. *Ferment of Turned Beer* (Pasteur).—"These are rods or filaments, simple or articulated into chains of variable length, of about 1 μ diameter.

A high power shows them divided into a series of shorter rods, scarcely born, not yet mobile at the articulations, which are scarcely indicated."

2. *Micrococcus* of a beer, having a particular acidity, distinct from that of beer *piqué*, having an acetic odor. "It consists of grains resembling little spherical points jointed by pairs or in fours square" (Pasteur), etc.

§ 2.—RÔLE OF THE BACTERIA IN PUTREFACTION AND NITRIFICATION.

While in the fermentations which we have just passed rapidly in review, we have always been able to study, at least summarily, the chemical action of the different organisms, we are now about to find ourselves in presence of phenomena far more complex. We will have to consider a great number of these vegetables at work, without its being possible to assign to each its *rôle*, or to say what is its function. The agent of the nitric fermentation has not as yet even been seen, and it is only by analogy that we class this nitrification with the true fermentations.

It is not only because of the obscurity which still exists in regard to a great number of peculiarities of these two phenomena, that we have united them in the same study. From the point of view of the circulation upon the surface of our globe of the elements essential to the constitution of organisms, they play an analogous *rôle*, although opposite the one to the other.

Let us consider, for example, nitrogen in plants. This element, of which the atmosphere is the reservoir, does not enter directly into combination, as does oxygen, with the other elements which with it are to constitute the immediate principles of the tissues. The chemical properties of nitrogen may be characterized in two words, — great resistance to entering into combination when it is free, and great facility, on the contrary, in passing from one combination to another when once it has associated itself with other elements.

The circulation of nitrogen in a state of combination upon the surface of the globe is also an interesting question of general physics, as well as the circulation of carbonic acid, of water, and of the air.

Let us seek to sketch the march of this circulation.

Whence comes the ammonia which is found in the sea, in the clouds which come to us from equatorial regions, in the dust of the air? The only known source is the fermentation of organic matters out of reach of the oxygen of the air. It is to this sort of fermentation that we owe the formation of peat and the immense masses of combustible minerals which have formed during nearly all the geological periods. We see this sort of fermentation develop itself when we expose an organic liquid to the air, but only in the inferior part of the liquid, the oxygen which is dissolved near the surface being arrested in the superficial zone, where a very different fermentation occurs.

The latter is essentially oxidizing; the material is almost completely burnt, forming water and carbonic acid; at the inferior part, on the contrary, a reduction is produced so energetic that hydrogen is disengaged. The metallic sulphates are there transformed into sulphites, and even crystals of sulphur are sometimes found (see the history of the *Beggiatoa*, page 91).

We see then the source of the ammonia, which, distributed upon the soil by the winds and the rains, becomes a powerful fertilizer. Now, vegetables do not absorb nitrogen under the form of ammonia, but under the form of nitric acid. How is this transformation of ammonia into nitric acid effected? The observations of Erdmann, Mensel, and T. Phipson show that in the phenomena of destructive putrefaction, nitric acid, far from being produced, is on the contrary reduced to the state of nitrous acid; on the other hand, Th. Schlœsing and A. Müntz conclude from their experiments that in the putrefactions essentially oxidizing produced by *Penicillum glaucum*, *Aspergillus niger*, *Mucor mucedo*, etc., there is no formation of nitric acid. But, according to these authors, nitrification is a special phenomenon which takes place in every soil sufficiently loose to permit a free circulation of air, and of which the agent is a micro-organism. This organism has not yet been perceived, it is true; and it is evident that it would be difficult to seek and observe, because of its peculiar situation.

But the action of chloroform upon nitrification tends to prove that the agent of this process is truly an organized ferment. Indeed, chloroform, this anæsthetic, suspends nitrification, and seems even to kill the ferment.

Leaving, then, this phenomenon, but little known, we may distinguish in the agents of putrefaction, or more generally of fermentation, two groups of micro-organisms, — one oxidizing, the other reducing.

The first are observed upon the surface of liquids undergoing putrefaction. We may distinguish a great number of forms, — *Bacterium termo*, *Monas crepusculum*, *Spirillum*, etc. We ought also to include *Mycoderma aceti*, which, like the others, vegetates on the surface of liquids, and a great number of organisms of which we cannot speak here.

The second are met, on the contrary, in the interior of liquids or of fermentable bodies; they are analogous to the butyric and lactic ferments, and perhaps to the other agents of diseases of wine and beer previously enumerated.

En résumé, the little beings which we have been considering have an important *rôle:* they cause the return of dead organic matter to the atmosphere and to water.

"Without them, organic matter, even exposed to the air, would not be destroyed or would be transformed with extreme slowness, in consequence of a slow combustion produced by oxygen. With them, on the contrary, its destruction takes a rapid march and becomes complete. If, then, the equilibrium is maintained between living nature and dead nature, if the air has always the same composition, if the waters are always equally fertilizing, it is thanks to the infinitely minute agents of fermentation and putrefaction" (Duclaux).

But the *rôle* of bacteria is not limited to this. "They invade also the living organism," says Duclaux, "and bring in their attack this double character of infinite smallness in the apparent means and powerful destructive energy in the results. From this source come diseases of which medicine, not long since, did not know the cause, and which she only commences to refer to their veritable origin. For those who are *au courant* with the first steps which she has made in this new line of research, with the fecundity of her first glimpses, with the richness of her first results, it is not doubtful that she will soon succeed in demonstrating the parasitic nature of the gravest epidemic maladies"

PART THIRD.

TECHNOLOGY.

◆

PART FOURTH.

GERMICIDES AND ANTISEPTICS.

◆

PART FIFTH.

BACTERIA IN INFECTIOUS DISEASES.

◆

PART SIXTH.

BACTERIA IN SURGICAL LESIONS.

By DR. G. M. STERNBERG.

PLATE IX.

FIG. 1. — Micrococci from *bottom* of culture-solution (rabbit-*bouillon*) inoculated with blood of septicæmic rabbit, containing the same micrococcus in active multiplication, as shown in Fig. 3. Magnified 1000 diameters by Zeiss's $\frac{1}{18}$ in. hom. ol. im. objective. Methyl-violet staining.

FIG. 2. — The same micrococcus cultivated in chicken-bouillon, inoculated with human saliva. × 1000. Same objective and staining.

FIG. 3. — The same micrococcus as found in the blood of a rabbit, inoculated with normal human saliva. (See p. 234.) × 1000 diameters; Zeiss's $\frac{1}{18}$ in. objective. Methyl-violet staining.

FIG. 4. — Micrococci from culture-solution (chicken-bouillon) inoculated with gonorrhœal pus. × 1000 diameters; Zeiss's $\frac{1}{18}$ in. objective. Methyl-violet staining.

FIG. 5. — Micrococci from urine passed into a sterilized glass vessel and allowed to stand five days, (covered with a watch-glass and bell-glass; Lister's apparatus, Fig. 5, p. 176,) believed to be identical with those shown in Fig. 3, and with *Micrococcus ureæ*, Cohn. (See description on p. 75.) × 1000 diameters; Zeiss's $\frac{1}{18}$ in. objective. Aniline brown staining.

FIG. 6. — Micrococci from culture-solution (malt-extract,) inoculated with normal human saliva, probably identical with the preceding; showing multiplication in two directions. × 1000, by Zeiss's $\frac{1}{18}$ in. objective. Aniline brown staining.

FIG. 7. — *Micrococcus ureæ*, from alkaline urine, showing formation of "chaplets,"— torula-chains, — by division in one direction only. × 1000, by Zeiss's $\frac{1}{18}$ in. objective. Aniline brown staining.

PLATE IX.

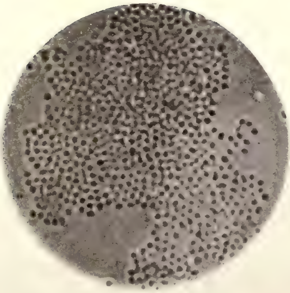

FIG. 1.

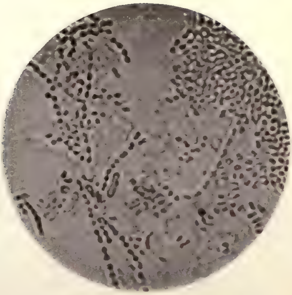

FIG. 2.

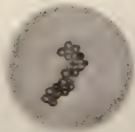

FIG. 4.

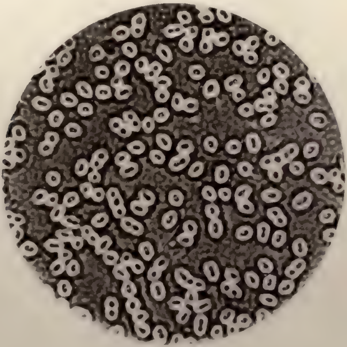

FIG. 3.

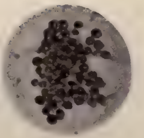

FIG. 5.

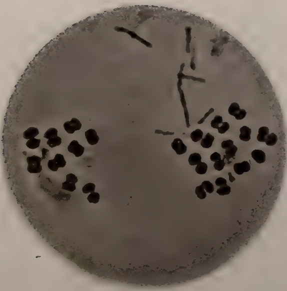

FIG. 6.

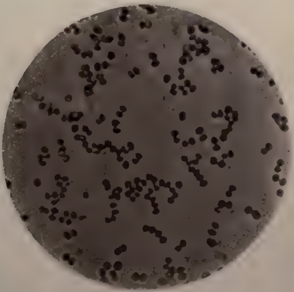

FIG. 7.

PART THIRD.

TECHNOLOGY.

OWING to their minute size and the difficulties attending their study, the Bacteria received but little attention from naturalists prior to the discovery by Davaine of the anthrax bacillus (Communicated to the French Academy of Sciences in 1863).

Since this date, very great progress has been made in our knowledge of these minute plants; and this progress has been due, to a considerable extent, to the labors of physicians rather than to those of botanists, who, as a rule, have been inclined to make light of the importance attached to this and subsequent discoveries relating to the presence of parasitic micro-organisms in the blood or tissues of man and the lower animals while suffering from certain infectious diseases. We are greatly indebted, however, to the German botanists, Cohn and Nägeli; and to the distinguished French chemist Pasteur must be awarded the foremost place among those who have contributed to our knowledge in this direction.

As in other branches of science, progress has to a great extent been dependent upon improvements in technique. These relate especially to methods of cultivation, and to the staining, mounting and photographing of bacterial organisms.

The object of the present chapter is to give as concise an account as possible of the technology as at present perfected, and as employed by the most successful modern investigators.

§ 1. — METHODS OF CULTIVATION.

For the solution of many problems relating to the life-histories and physiological functions of the various species of Bacteria, it is essential that a "pure culture" be obtained and maintained through successive generations by the inoculation of fresh portions of a suitable culture-medium. Evidently this requires not only pure stock to commence with, but also a culture-medium free from living organisms — *sterilized,* — and the exclusion of floating atmospheric germs.

Methods of Obtaining Pure Stock.—Various methods have been devised for the purpose of isolating a single species when mingled, as is commonly the case, with many others. Lister proposed to accomplish this by diluting the material containing a number of distinct species — e. g. a drop of human saliva or of broken-down beef tea which has been freely exposed to the air — with a sterilized fluid until there shall be an average of less than one living germ to each drop of fluid. If now we inoculate numerous separate portions of a sterilized culture-medium with a single drop, each, of this diluted stock, it is evident that some portions may receive no living seed, others may have germs of two or more species, and others may chance to have one or more germs of a single species. In the latter case, the multiplication of these germs under conditions which excluded the possibility of contamination from without would give us a pure culture of this particular species. So far as the writer is aware this method has not been employed, except in a limited number of experiments made by Lister himself in order to demonstrate its feasibility. No doubt it may be successfully employed, but it would involve a great expenditure of time, and success would probably be the exception and failure the rule, owing to the difficulty of estimating the exact amount of dilution required in the first instance, and because of the element of chance, which is an essential feature of the method.

The same result is accomplished more expeditiously by the method of Koch, the essential feature of which consists in using a solid sub-stratum as the culture-medium, upon which the mixed micro-organisms are distributed. A sufficient quantity of gelatine (3 to 5 per cent.) is added to a suitable culture-fluid to cause the mixture to jellify when cooled. While still warm, this gelatine culture-fluid is poured upon glass slides, to which it adheres when cool in the form of a semi-solid layer. Upon this the mixed bacteria are distributed by means of a needle, the point of which is lightly drawn across the surface, after having been charged with seed by dipping it into the stock-solution a biological analysis of which is desired — e. g. broken-down urine or beef tea. The different micro-organisms are distributed by this method along the track of the needle, and the subsequent multiplication of each germ *in situ*, when the slide has been left for a day or two in the culture-oven, produces a little collection of the particular species to which it belongs, which may be recognized under the microscope or even by the naked eye.

A pure culture is obtained by inoculating a sterilized culture-fluid with seed, transferred with due precautions, from one of these little masses formed along the track of the needle.

Another method which suggests itself, and will doubtless be found useful in certain cases, depends upon the difference as to reproductive activity manifested by different species of bacteria, and upon the fact that a culture-medium, or conditions as to temperature, favorable for the development of one species may not be for another. By taking advantage of these physiological peculiarities we may succeed in excluding all but a single form, by one or more culture experiments, notwithstanding the fact that our stock was impure at the outset. It is evident that if one species multiplies more promptly and rapidly than the others which are associated with it, it will soon be present in excess in a culture-fluid inoculated with the commingled species, and that by using this stock to start a second culture before other forms have time to multiply, repeating the operation if necessary through a series of cultures, we shall at last exclude all except the single species which has taken precedence by virtue of its rapid multiplication.

In the same way we may take advantage of conditions relating to the composition of the culture medium, and to the temperature at which it is maintained after inoculation with impure stock. When the conditions are most favorable for the development of a particular species, it is evident that this will take precedence over others with which it is associated. And it may happen that conditions extremely favorable for one are entirely unsuited for other species which, accordingly, do not multiply at all.

We have examples of this in the experiments which have been made upon living animals, which may be considered culture-experiments, in which the blood of the animal serves as a culture-fluid, and in which the temperature maintained is necessarily that of the species used in the experiment. Thus in the form of septicæmia in the mouse, which has been studied by Koch, a drop of putrid blood "containing bacteria of the most diverse forms irregularly mixed together," injected beneath the skin of the animal, gives rise to an infective disease characterized by, and dependent upon, the presence of a multitude of minute bacilli in the blood and tissues. In this case, it is evident that the conditions are favorable for the multiplication of this species, and not for the others associated with it in the drop of putrid blood introduced into the living culture-apparatus. This experiment enables us to secure a pure culture of this particular bacillus; for the smallest quantity of blood taken from the vessels of the animal, immediately after its death, contains it in abundance, and may be used to inoculate a sterilized culture-fluid. In the same way, if we inoculate a rabbit with a drop of human saliva, which contains a variety of bacteria, one species only multiplies freely and invades the blood of the animal, producing a fatal infectious disease. This is a micrococcus of oval form and having peculiar characters. (Fig. 3, Plate IX.) By introducing a little of the blood of a rabbit, just dead as the result of such an inoculation, into a sterilized culture-fluid, we obtain a pure-culture of this micrococcus, which may be maintained indefinitely through successive generations from culture-tube to culture-tube, or from rabbit to rabbit, thus showing that this micrococcus is a distinct species, as it "breeds true."

Having obtained pure stock by one of the methods mentioned, success in cultivating the species contained in it will depend upon the use of a suitable culture-medium, and the maintenance of favorable conditions as to temperature and a sufficient supply of oxygen, if required.

Natural Culture-Fluids. — The natural culture-fluids which are available for use are blood, milk, urine, and aqueous humor from the eye of one of the lower animals.

All of these have been used, and all may be obtained in a pure state from the living animal by adopting proper precautions.

Blood. — The observations of numerous experimenters prove that the circulating fluid in healthy animals is free from all bacterial organisms. To obtain a supply for experimental purposes it must be drawn directly from the vessels into a sterilized receptacle. This may be accomplished by means of a glass tube drawn out at each end to form a capillary tube, hermetically sealed at each extremity and thoroughly sterilized by heat. Such a tube is to be filled by exposing a superficial vein of sufficient size, and introducing one of the capillary extremities within the vessel through a very small orifice made through its walls. The end of the tube is to be broken off within the vessel, after which the outer end may also be broken, to allow the contained air to escape as the tube fills with blood. This will not be necessary, however, if a partial vacuum has been formed by sealing the capillary extremities in the flame of an alcohol lamp while the tube was still quite hot. Both extremities are sealed as expeditiously as possible as soon as the tube is withdrawn from the vessel. It is evident that to obtain a larger quantity of blood, a flask having two necks bent at a right angle and drawn out to form capillary tubes may be substituted for the simple straight glass tube. (See Fig. 1.)

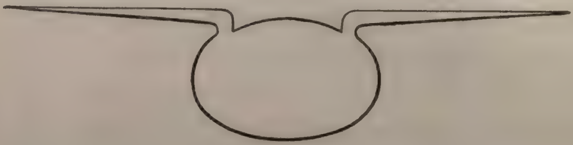

Fig. 1.

The color of the blood, due to the presence of the red corpuscles, and the fact that these elements, after a time, form a granular debris which might interfere with the recognition of minute micrococci, are objections to the use of this fluid in culture experiments. Blood-serum, however, is free from these objections, and is a valuable culture-medium. This may be obtained from a flask, like that shown in Fig. 1, by transferring the serum, after it has separated from the clot, to small culture-flasks like those described on page 179 (Fig. 5), by the method there detailed. To accomplish this, one of the arms of the larger flask is broken off to admit the capillary extremity of the smaller one. By skilful manipulation a number of these may be filled with transparent serum with but little chance of contamination by floating atmospheric germs.

Blood-serum obtained without these special precautions may also be used by resorting to the method of Koch for sterilizing it subsequently to its separation from the clot. This is accomplished by introducing it into test-tubes from which atmospheric germs are excluded by a plug of cotton, or into hermetically sealed culture-flasks, like those described on page 179, and exposing it for an hour daily to a temperature of 58° C. (136.4° Fahr.) for a period of six days. This method insures the destruction of living germs contained in the blood-serum without coagulating the albumen, which would destroy its value as a culture-fluid. If a solid culture-medium is desired, the blood-serum is subsequently subjected to a temperature of 65° C. (149° Fahr.) for several hours. A solid, transparent, jelly is produced by this method, which is the material upon which Koch cultivated the tubercle bacillus in his experiments relating to tuberculosis.

Milk.—The experiments of Lister, Roberts and Cheyne have demonstrated that milk, as it exists in the udder of the cow, is free from the germs of fermentation or putrefaction, and may be preserved indefinitely without undergoing change, if proper precautions are taken to introduce it into sterilized flasks without contamination by organisms detached from the external surface of the body of the animal or by floating atmospheric germs. It is difficult to accomplish this, however, and in practice it will be found that milk, although a suitable culture-fluid for various organisms, is not commonly available, owing to the difficulty of obtaining it from its source free from contamination, and to the fact that it is a difficult fluid to sterilize.

Urine.—Pasteur, Lister, the present writer, and several other experimenters have succeeded in obtaining urine, directly from the bladder, free from bacterial contamination, and which, consequently, did not undergo any change from being kept, although exposed freely to the air—*filtered*—and to a temperature suitable for inducing the different forms of fermentation which this fluid undergoes when no precautions are taken to exclude the micro-organisms to which these changes are due.

In man, and doubtless in the lower animals also, the orifice of the urethral canal is constantly infested with bacteria of different species, whereas the deeper portion of the canal and the bladder are quite free from them. This is proved by microscopical examination, and by the fact that urine free from bacteria may be obtained by taking the precaution to destroy those located in the vicinity of the meatus urinarius by means of a suitable disinfectant.

The writer has on several occasions repeated with success the experiment of Lister, the essential feature of which is the thorough cleansing and disinfection of the urethral canal by means of a solution of carbolic acid (5 per cent). The glans should also be washed with the same solution; after which the urine is passed into a glass flask or test-tube which has been sterilized by heat. This is at once closed with a plug of cotton.

Urine has been extensively used as a culture-fluid, and is well suited for the development of many species of bacteria; and especially for the micrococcus, which has been shown by Pasteur to be the cause of the alkaline fermentation which ordinarily occurs in this fluid during warm weather, within a few hours after its escape from the bladder. It must be remembered, however, that decomposition of urea into carbonate of ammonia is also effected by heat, and that, consequently, the composition and reaction of this fluid is changed by boiling. For this reason its sterilization by heat is objectionable for certain experiments, and it will be necessary to obtain it from the bladder free from bacterial contamination, by the expedient above mentioned (method of Lister), or by means of a sterilized catheter attached to a germ-proof receptacle, as recommended by Pasteur.

Aqueous humor, obtained from the eye of one of the lower animals, recently dead, is a sterile albuminous fluid which has been utilized, especially by the earlier investigators, as a culture-medium. The method of operation has commonly been to place a drop of this fluid, obtained from the eye through a sterilized canula, upon a perfectly clean cover-glass, and to invert this over a shallow glass cell the margin of which has been wet with olive oil, or with a liquid cement of some kind. This serves to attach the cover and to exclude atmospheric organisms. The drop of fluid is inoculated by means of a needle, the point of which has been dipped into the stock-solution containing the particular organism which it is proposed to cultivate.

This method is especially useful when the development of an organism is to be studied by continuous observation; for the slide supporting a culture-cell made in this way may be placed upon the stage of the microscope, and bacteria in the drop of fluid may be observed with high powers through the thin glass cover. This method does not, however, offer as perfect security as regards the exclusion of extraneous organisms as is desirable, and it has generally been abandoned for the methods to be described later, in which a considerable quantity of fluid, enclosed in a germ-proof receptacle, is used. In this case a microscopical examination of the contained organisms requires that a small portion of the culture-fluid be withdrawn from the culture-flask, and continuous observation would be impracticable.

Artificial Culture-Fluids. — The culture-fluids which have been most extensively used in investigations relating to the physiology and life-histories of the various species of bacteria are infusions of animal and vegetable substances, such as beef, mutton, chicken, fish, gelatine, turnip, potato, cucumber, hay, malt, etc., etc. These infusions, as a rule, do not require to be very concentrated, and they should be as transparent as possible, as the slightest opacity from suspended particles, albuminoid or inorganic, may interfere with the detection by the naked eye of changes in the fluid due to the development of bacteria, and with the recognition of these organisms upon microscopical examination. It sometimes occurs that an infusion of beef or of chicken, which has been carefully filtered and is quite transparent, becomes opalescent from the coagulation of a minute quantity of albuminoid material as the result of the operation of sterilization. I have found this opalescence difficult to remove by filtration. It is objectionable, but could hardly be mistaken for the opalescence, or milky opacity, which results from the breaking-down of an infusion of this kind, and with due care the experimenter is not likely to be deceived, especially if he retains a portion of the sterilized fluid for comparison with that used in his culture experiments.

Nitrogen, which is an essential element of the protoplasm of bacterial organisms, is supplied by the albumen of animal or vegetable origin which remains in solution in the above-mentioned culture-media. But this element can also be appropriated when present in the form of ammonia, or of one of the salts of ammonia in combination with a vegetable acid.

Culture-fluids may therefore be made which are suitable for the development of numerous species of bacteria, by adding to distilled water a small quantity of a salt of ammonia, together with certain mineral salts, as in the formula of Mayer, given on page 113. Pasteur's solution contains ten per cent. of sugar candy and a fraction of one per cent. of ashes of yeast. (See p. 112.)

Sterilization of Culture-Fluids.—Heat is the agent most available for the sterilization of culture-fluids, as chemical reagents which would accomplish the same result would also, by their presence in the fluid, prevent the development of organisms introduced for the purpose of cultivation. It would doubtless be possible to sterilize a fluid by means of a chemical reagent — a mineral acid for example — and subsequently to neutralize the germicide agent — e. g. by lime or magnesia. But in practice it will be found that no other method is likely to give as satisfactory results as that commonly employed; which consists in subjecting the fluid, enclosed in a germ-proof receptacle, to a temperature which insures the destruction of the vitality of contained organisms.

The earlier experimenters assumed that a boiling temperature must be fatal to the minute organisms developed in organic infusions; and this false assumption furnished a foundation for the belief, entertained by some of them, that bacteria might appear in such fluids by heterogenesis. The assumption has been proved to be false by the experiments of Pasteur, of Tyndall and of many others, and it is now known that the reproductive spores, of endogenous formation, which are developed in certain species, may resist a temperature considerably above the boiling-point of water. (See p. 119.) The writer, while conducting a series of experiments in the biological laboratory of Johns Hopkins University, during the summer of 1881, was greatly troubled by the fact that the laboratory was infected by the spores of a species of bacillus, which developed in little islands on the surface of his culture-fluids, even when they had been boiled for an hour or more. To destroy the spores of this bacillus, it was necessary to resort to the use of a bath of paraffine, or of concentrated salt-solution, by means of which a temperature of 105° C. was secured. This temperature, maintained for half an hour to an hour, proved effectual in the destruction of these ubiquitous spores.

Prolonged boiling will doubtless destroy the vitality of the most refractory spores; but the exact time which is required to secure success in every case has not been determined. In practice, it will be found best to keep on the safe side, as the loss of time and material which results from imperfect sterilization is annoying, and mistakes may arise from a false confidence in the success of the operation. To avoid these, it is always best to test culture-fluids in the culture-oven for several days before using them for any experiment.

The maintenance of a boiling temperature at intervals for a day or two is more effectual than the same amount of continuous boiling. Pasteur has shown that an alkaline fluid is more difficult to sterilize than one having an acid reaction. The vitality of bacteria in active growth is destroyed by a comparatively low temperature. Thus Chauveau has recently made the statement (C. R. Ac. des Sc., t. XCIV. p. 1694), that the anthrax bacillus is killed (in blood) by exposure for nine or ten minutes to a temperature of 54° (129.2° Fahr.). According to Frisch, *B. termo* is killed by a temperature of 45° to 50° (113° to 122° Fahr.) — time of exposure not given. The writer has fixed the thermal death-point of the micrococcus of induced septicæmia in the rabbit at 60° (140° Fahr.), the time of exposure being ten minutes; that of *Micrococcus ureae* was found to be the same.

The method adopted by Koch for the sterilization of blood-serum for his experiments with the tubercle bacillus has already been mentioned (p. 165). This method depends for success upon the fact that the temperature employed, 58°, is sufficient to destroy growing bacteria, and that in the intervals between the daily heating for one hour the spores have an opportunity to germinate, and are killed by the subsequent heating. The writer has not been successful in sterilizing milk by this method, and has recently lost the greater portion of a batch of tubes containing blood-serum, carefully treated according to Koch's directions, from the development of *Penicillium glaucum* upon the surface of the jellified serum. The spores of this fungus were evidently very abundant in the laboratory at the time the serum was introduced into these tubes, which had been well sterilized by heat and were thoroughly protected by cotton wadding tied over the mouth of each, with the additional precaution of covering this with a piece of sheet-caoutchouc secured by a rubber band. No doubt the unusual abundance of the spores of Penicillium was due to the disturbance of the dust upon a lot of books which were taken down from an upper-shelf by my assistants, shortly before the blood-serum was decanted and introduced into the culture-tubes. According to Pasteur, the spores of Penicillium and other common mucedines are not destroyed by a temperature of 120 to 125° C (248–257° F.), *in the absence of moisture.*

Culture Tubes and Flasks. — Glass tubes or flasks are used as germ-proof receptacles for the sterilized culture-fluids mentioned. Ordinary test-tubes are commonly employed, and are useful for many purposes. They should be thoroughly heated in an oven, or in the flame of an alcohol lamp, just before the fluid is introduced, to destroy all germs adhering to their inner surface. The culture-fluid may be sterilized before or after its introduction into these tubes. In the former case, the operation must be performed expeditiously, in as pure an atmosphere as possible; and the mouth of the tube is to be closed at once with a plug of cotton-wool. It is evident that this method admits of the entrance of floating atmospheric germs while the tubes are being filled, and, therefore, that a certain proportion are likely to break down. The percentage of failures will depend upon the skill of the operator and upon the purity of the atmosphere in which the operation is performed. The liability to failure from contamination by floating germs is not, however, as great as is commonly imagined; and experience proves that contact with instruments or surfaces — e. g. the lip of the vessel from which the culture-fluid is poured — which are not perfectly pure, is a more frequent cause of the breaking-down of the culture-fluid.

Sterilization of the culture-fluid after its introduction into the tubes, offers greater security, and the following method of manipulation is recommended: Test-tubes, or wide-mouthed bottles having a capacity of half an ounce or more, are washed clean, and the mouth of each is covered with several layers of cotton-wadding. This is secured in position by means of a strong linen thread, or a piece of copper wire, tied about the neck. The wide-mouthed bottles have the advantage of being less fragile, and of standing without support. They are especially useful for receiving a solid culture-medium, such as gelatine solution or jellified blood-serum, as the surface exposed is greater than when test-tubes are employed. The only disadvantage attending the use of bottles is their liability to break when heated in a water-bath; but this will not happen when Koch's method of sterilization at a low temperature (140° Fahr.) is employed. The tubes, or wide-mouthed bottles, are next placed in an oven and subjected for an hour or more to as high a temperature as the cotton caps will bear without being scorched — about 300° Fahr. They are then cooled, and the culture-fluid is introduced, without removing the protective cotton-cap, through a little funnel having a long and sharp-pointed neck, which is pushed through the layers of cotton-wadding, either directly or after making a small orifice with a sharp-pointed instrument. Usually but one or two drachms of fluid will be required in each tube. This must be sterilized by heat, after its introduction to the culture-tube, unless it is introduced directly from a germ-proof flask with a slender neck, such as the writer recommends for the preservation of culture-fluids in bulk (Fig. 5, p. 179). In this case, the slender neck of the flask is passed through the flame of an alcohol lamp, to destroy germs which may have settled upon its outer surface; and the hermetically sealed extremity is broken off with forceps which have also been recently heated. The flask is then inverted, and the capillary neck is passed through the opening in the protective cap of a culture-tube. A sufficient quantity of fluid is then transferred by the application of gentle heat to the base of the inverted flask. (See Fig. 2.) Care must be taken not to wet the protective cotton with the culture-fluid; and immediately after this has been introduced, the orifice in the cotton wadding is closed by placing two more layers of the same material over those which had previously been secured to the neck of the bottle or tube. This outer protective layer may be conveniently secured in position by means of a rubber band which admits of its being quickly removed for the purpose of introducing the bacteria which it is proposed to cultivate, or of extracting a drop of fluid for microscopical examination. This is accomplished by means of a capillary tube which has been sterilized by heat just before it is used, and which is introduced through the small opening in the inner layers of the cotton cap. When tubes or bottles prepared in this way are set aside for a considerable time, or when the free admission of oxygen to the interior is not considered necessary, it is well to cover the cotton cap with a piece of thin sheet-caoutchouc, secured by means of a rubber band. This serves to protect the cotton cap

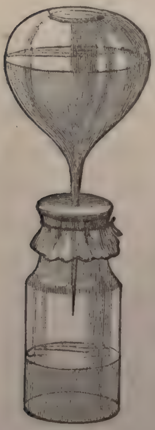
Fig. 2.

from dust, and the contained fluid is less liable to contamination when the outer layer of cotton-wadding is removed for any purpose. It is well to carbolize the cotton-wadding used for the outer protective cap, as recommended by Lister. This is done by soaking it in a solution of one part of crystallized carbolic acid in one hundred parts of anhydrous ether, after which it is allowed to dry.

Lister has shown that organic infusions may be kept indefinitely, without undergoing change, in a wine-glass covered first with a watch-glass, and then with a glass shade as shown in Fig. 3. The apparatus, as arranged in the figure, is purified by being introduced into a hot oven; and after it has cooled, the sterilized fluid is introduced from a large, double-necked stock-bottle, seen in Fig. 4. To do this, the cotton cap is removed from the nozzle of the stock-bottle, and the half of a rubber ball, having an opening in the centre, is attached to its extremity. This rubber hemisphere, which has been previously sterilized by soaking it in a strong solution of carbolic acid, serves the purpose of covering the mouth of the wine-glass when the glass cover — watch-glass — is removed.

Culture-Flasks used by the Author. — The writer described, in a paper read at the meeting of the American Association for the Advancement of Science, in August, 1881, a method of conducting culture-experiments which he has found extremely satisfactory, and which has the advantage of assuring the greatest possible security from contamination by atmospheric germs. The culture-flasks employed contain from one to four fluid drachms. "They are made from glass-tubing of three or four tenths inch diameter, and those which the writer has used in his numerous experiments have all been home-made. It is easier to make new flasks than to clean old ones, and they are thrown away after being once used. Bellows, operated by the foot, and a flame of considerable size — gas is preferable — will be required by one who proposes to construct these little flasks for himself. After a little practice, they are rapidly made; but as a large number are required, the time and labor expended in their preparation is no slight matter. . . . After blowing a bulb at the extremity of a long glass tube, of the diameter mentioned, this is provided with a slender neck, drawn out in the flame, and the end of this is hermetically sealed. (See Fig. 5.) Thus one little flask after another is made from the same piece of tubing, until this becomes too short for further use.

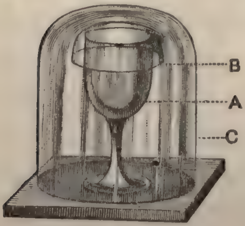

Fig. 3.

a, wine-glass; *b*, glass cover (watch-glass); *c*, bell-glass, supported by a square glass plate.

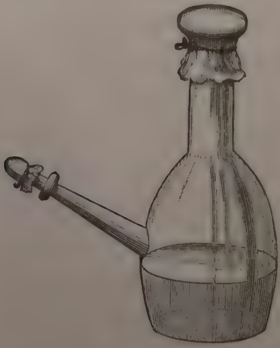

Fig. 4.

"To introduce a culture-liquid into one of these little flasks, heat the bulb slightly, break off the sealed extremity of the tube and plunge it beneath the surface of the liquid (see Fig. 6). The quantity which enters will of course depend upon the heat employed, and the consequent rarefaction of the enclosed air. Ordinarily the bulb is filled to about one third of its capacity with the culture-liquid, leaving it two thirds full of air, for the use of the microscopic plants which are to be cultivated in it.

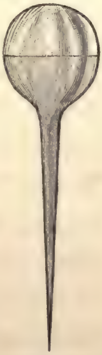
Fig. 5.

"It is best not to trust to the sterilization of the culture-liquid previously to its introduction into the flasks; for, however great the precautions taken, many failures would be sure to occur, as the result of contamination by atmospheric germs during the time occupied in the manipulations. Sterilization is therefore effected by heat after the fluid has been introduced and the neck of the flask hermetically sealed in the flame of an alcohol lamp.

"This may be accomplished by boiling for an hour in a bath of paraffine or of concentrated salt solution, by which a temperature considerably above that of boiling water is secured. The writer is in the habit of

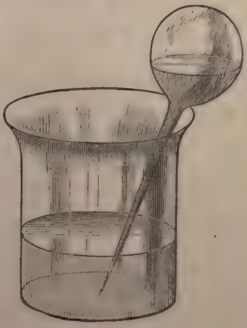
Fig. 6.

preparing a considerable number of these flasks at one time, and leaving them, in a suitable vessel filled with water, for twenty-four hours or longer upon the kitchen stove. Here the water-bath is kept boiling at intervals, and the contents of the flasks can scarcely fail of being subjected to a temperature of 212° Fahr. for eight or ten hours. When the time is less than this, failures in sterilization are likely to occur, and it is always best to keep on the safe side. The flasks are next placed in a culture-oven for two or three days, at a temperature of 35 to 38° (95 to 100° Fahr.), to test the success of the previous operation, — sterilization. If at the end of this time the contents remain transparent, and no film — *mycoderma* — has formed upon the surface of the liquid, the flasks may be put aside for future use, and can be preserved indefinitely.

"To inoculate the liquid contained in one of these little flasks with organisms from any source, the end of the tube is first heated, to destroy germs attached to the exterior; the extremity is then broken off with sterilized — by heat — forceps; the bulb is very gently heated so as to force out a little air; and the open extremity is plunged into the liquid containing the organism to be cultivated. The smallest quantity of this is sufficient, and as soon as the inoculation is effected, the end of the tube is again sealed in the flame of an alcohol lamp. A little experience will enable the operator to inoculate one tube from another; to introduce a minute quantity of blood containing organisms directly from the veins of a living animal; to withdraw a small quantity of fluid from the flask for microscopical examination, etc., without any danger of contamination by atmospheric germs."[1]

A larger flask than those above described, having its neck drawn out in the same way, will be found the most satisfactory receptacle in which to preserve a quantity of stock solution from which to fill the smaller flasks as required. It is well not to attempt to preserve too great a quantity of the various organic infusions used in experimental work of this kind, in a single flask; as there is greater danger of the breaking down, and consequent loss, of the stock, when a vessel is frequently opened for the purpose of withdrawing a portion of its contents. It is best therefore to use a number of flasks of moderate size, rather than a single large one. There is always a saving of time and labor, when extensive experiments are contemplated, in preparing a considerable quantity of the various culture-fluids at one time, so that there may be a sufficient stock on hand in the laboratory to enable the experimenter to proceed without delay with any series of experiments he may have in view. The writer keeps constantly on hand a supply of the little flasks already described, charged with sterilized urine, beef-tea, chicken *bouillon*, hay infusion, Cohn's fluid, etc., and would recommend others who may be inclined to pursue experimental investigations relating to the bacteria to provide themselves in the same way. For a reserved supply of these and other culture-fluids, flasks containing from two to four fluid ounces will be found of a convenient size. The necks of these flasks are to be drawn out in a powerful flame, so as to form a slender tube the extremity of which can be easily fused in the flame of an alcohol lamp, and which is long enough to permit of its being broken off at the end and resealed several times. The fluid is introduced into these flasks exactly as directed for the smaller ones, viz., by applying heat to the body of the flask, so as to rarefy the enclosed air, and plunging the extremity of the slender neck of the flask, inverted, beneath the surface of the fluid contained in a suitable vessel. These flasks are to be hermetically sealed and sterilized exactly as was directed for the smaller ones. Each flask should have attached to it a label showing the character of its contents and the date of sterilization.

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[1] Extract from a paper by the Author on "The Germicide Value of certain Therapeutic Agents." The American Journal of the Medical Sciences, No. CLXX., n. s., pp. 321-343.

Culture-Oven. — As culture experiments are commonly conducted at a constant temperature, it is necessary to have a receptacle for the culture-tubes and flasks which can be heated artificially to any desired point, the temperature being regulated by a thermostat.

A rectangular copper vessel, having double walls to contain water, enclosing an air-chamber, will be found most suitable for this purpose. When the space between the double walls is filled, the air-chamber is surrounded with water on all sides, except that through which access to it is obtained. This side is closed by a swinging or sliding door. If the oven is of considerable size, it is well to have one or more adjustable shelves in the interior, upon which tubes and flasks may be placed, as well as upon the floor. A suitable aperture at the top admits the thermostat to the water-bath, and another aperture serves for the introduction of more water when required. A third aperture, through the centre of the upper side of the oven, leads to the air-chamber, and admits of the introduction of a thermometer, the index of which can be read outside while the bulb is inside of the oven. In a well-equipped laboratory several of these culture-ovens will be required, as experiments conducted at different temperatures will often be under way at the same time.

The most convenient way of heating an oven of this kind is by the use of gas and of a Bunsen or other burner, which insures the complete combustion of the carbon. When gas is used, the thermostat described below, well known in chemical laboratories, may be employed.

Thermostat for Gas (Fig. 7).—The elongated glass bulb *a* contains a certain quantity of mercury below, and air above. When the air is expanded by heat, the mercury rises through the tube *c*, which passes through the perforated cork *d*, and flows into the space above this cork. The tube *e* is connected by a piece of rubber tubing with a gas-jet, and the gas continues to pass through the tube *f* to the Bunsen burner, unless arrested by the rising of the mercury, which acts as a valve to close the lower extremity of the tube *e*. This tube is adjustable through the cork *i*, and it is evident that the temperature at which the gas supply is shut off will depend upon the position of its lower extremity. A minute aperture in the side of the tube *e* permits a small quantity of gas to flow to the burner, so that the flame may not be entirely extinguished when the extremity of this tube is closed by the rising of the mercury. There is danger, however, when but a small amount of gas is admitted to a Bunsen burner that the flame may be extinguished by currents of air. It will therefore be found best, in practice, to close this aper-

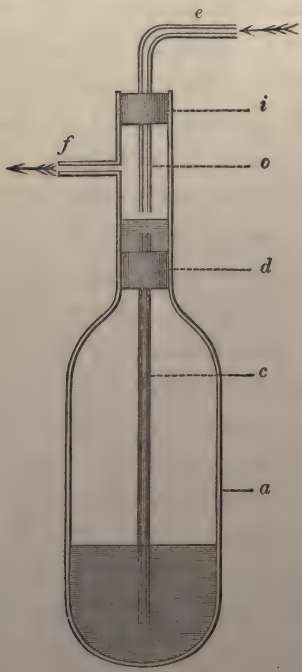

Fig. 7.

ture, and to have a small constant jet of gas at the side of the burner, in a position to relight the gas coming through the thermostat to the burner when the valve is opened by the falling of the mercury. The gas for this side jet does not pass through the burner or the thermostat.

When the experimenter is so situated that he cannot obtain a supply of gas, the problem of regulating temperature is not quite so simple; but the result may be accomplished by the use of a magneto-electric thermostat invented by the writer some years since.

The regulating thermometer, Fig. 8, may be made as in the thermostat just described; but, instead of a tube conveying gas, the mercury, when it rises through the tube *c* to the space above the cork *d*, meets at a certain point — adjustable — the insulated platinum wires *e* and *f*, completing an electric circuit. A constant battery is required, — a single cup is sufficient, — and an electro-magnet, the lever of which is made, by some simple contrivance, to cut down the flame of the kerosene or alcohol lamp used as a source of heat.

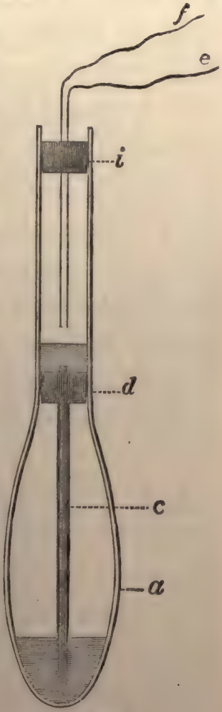

Fig. 8.

This electro-magnetic regulator may also be used with gas, when great accuracy is required, by employing the valve shown in Fig. 9, which was invented by the writer for this purpose several years since.

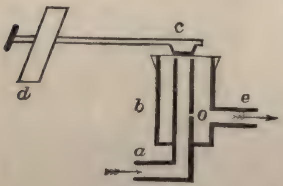

Fig. 9.

The bent tube *a* is connected with the gas supply by a piece of rubber tubing. The upright arm of this tube is enclosed in a larger tube *b*, having an outlet *e*, which is connected with the burner. The upper end of this larger tube is closed by means of a piece of sheet-rubber, and when this is depressed by means of the lever *c*, the flow of gas through the valve is arrested. The lever *c* has attached to it the armature *d*, and is operated by an electro-magnet under the control of the regulating thermometer. To prevent the flame at the burner from being entirely extinguished every time the valve is closed, a small aperture *o* is made in the upright arm of the bent tube *a*.

§ 2. THE RECOGNITION OF BACTERIA. — The breaking down of a culture-fluid, either as the result of inoculation or of accidental contamination, may commonly be recognized by the naked eye. The fluid, previously transparent, may become opalescent or milky in appearance, from the presence of a multitude of bacteria distributed through it; or we may observe a pellicle upon the surface, while the fluid below remains transparent; or, if some time has elapsed, the micro-organisms, having exhausted the pabulum necessary for their development, may have settled to the bottom, where they form a white pulverulent precipitate, while the fluid above is transparent. In the latter case, a milky appearance is produced by shaking the tube so as to distribute the organisms throughout the fluid.

There is usually no difficulty in recognizing, by means of the microscope, the minute unicellular plants to which this change in our culture-fluid is due. But for this purpose it will often be necessary to use comparatively high powers, — e. g., a good one-tenth inch objective, — and to resort to the use of staining reagents. For information relating to the optical and chemical tests by which bacteria are to be distinguished from inorganic substances, and from albuminous or fatty granules, etc., the reader is referred to Part First of the present volume, which treats of their morphology, and especially to the remarks in the second chapter, pages 49–53.

Motile bacteria are at once recognized as living organisms; but care must be taken not to mistake movement due to currents in the fluid, or the molecular motion — brownien — which minute particles undergo when suspended in a fluid, for a vital movement. This is to be distinguished by the fact that the movements are vibratory, and do not result in a change in the location or relative position of the moving particles. Bacteria which have exactly the same refractive index as the fluid in which they are immersed, are invisible; but if endowed with active movement, they may be detected by the disturbance they cause among motionless objects which happen to lie in their course. Thus the septic vibrio of Pasteur is so slender and transparent as to be almost invisible; but when present in the blood of a septicæmic rabbit, its vigorous serpentine movements are marked by a displacement of the blood globules, which it moves as a serpent moves the grass in which it is concealed. This septic vibrio I have found in the blood of rabbits, victims of my experiments in New Orleans during the summer of 1880.

The use of staining reagents is indispensable for the recognition of these extremely transparent or extremely minute species. Their value has recently been demonstrated by Koch, in a most striking manner, by the discovery of a specific bacillus in the lungs and sputum of patients suffering with pulmonary consumption, which had escaped the observation of pathologists and microscopists up to the time of his announcement of its presence and peculiar color-reaction.

§ 3. STAINING BACTERIA.—By far the most useful staining reagents are the aniline dyes, first recommended by Weigert. Previously to the introduction of this method, hæmatoxylin had been used to some extent, but did not give very satisfactory results, as "it does not stain rod-shaped bacteria at all, and only colors the spherical so slightly as to prevent their certain recognition when isolated" (Koch). The aniline colors most used are the methyl-violet, aniline-brown, fuchsin, and methyl-blue.

An aqueous solution of methyl-violet is perhaps the most generally useful staining fluid; and in the violet ink sold by the stationers we have a solution ready made, which answers every purpose. It usually requires to be filtered. The mode of operating is as follows: The fluid containing the bacteria to be stained is spread in as thin a layer as possible, and allowed to dry, upon a thin glass cover. The drying may be hastened by passing the cover-glass, held in forceps, through the flame of an alcohol lamp. A drop or two of the staining-fluid is then poured upon the cover-glass, and after being left a short time is washed away by a gentle stream of water, or by agitating the cover in a glass of clean water. Usually one or two minutes is sufficient time to ensure the staining of the bacteria attached to the cover. For immediate examination, it is now only necessary to place the cover on a glass slide over a little drop of distilled water. It is better, however, to support the margin of the cover by means of a circle of white zinc cement, turned in the centre of the slide. This prevents the bacteria from being detached by contact with the slide. If the object is to make a permanent preparation, a drop of some preservative fluid is placed in the shallow cell formed by the circle of cement. A saturated solution of acetate of potash, or a weak solution of carbolic acid (one per cent), or camphor water, may be used for this purpose. The surplus fluid is removed with blotting paper, and another circle of cement is turned about the margin of the cover to hermetically seal the cell. Permanent preparations may also be made by mounting in Canada balsam. In this case, the cover-glass is allowed to dry after staining, and may be treated with alcohol and oil of cloves, although this is usually unnecessary; and too long an exposure to the action of these agents is likely to remove the color from the bacteria.

To demonstrate the presence of bacteria in the tissues, the following method, devised by Weigert, is strongly recommended by Koch: —

> "The objects for examination are first hardened in alcohol. The sections made from these are allowed to lie for a considerable time in a pretty strong watery solution of methyl-violet. They are then treated with dilute acetic acid, the water removed by alcohol, cleared up in oil of cloves, and mounted in Canada balsam. . . .
>
> "This is of course only a general outline of the method; for the individual tissues, and more especially the different forms of bacteria, show so great a variety of result from such treatment that it would be impossible to lay down rules which would be universal and which would apply to every case. For many objects fuchsin is best adapted; for others the methyl colors are more suitable. Among these latter there exists such a difference in the staining power that the sections must lie in one solution only a few minutes, in another several hours. . . .

"The strength of the acetic acid solution is not of much consequence. The best solution is one containing only a small percentage of the acid, and it is well not to allow it to act too long. The other manipulations, such as the removal of water, clearing up, and mounting, are exactly the same as in the preparation of other microscopic specimens. One must avoid leaving the sections too long in alcohol or oil of cloves; otherwise the staining material will be washed out by these fluids."[1]

The method above described brings to view the larger forms of bacteria which may be distributed through the tissues; but, according to Koch, the smaller forms may not be distinguished, although deeply stained, and require for their demonstration a special form of illuminating apparatus, which brings out the "color picture," while details of structure are to a great extent lost (*l. c.* p. 27). The illuminating apparatus of Abbe, made by Zeiss of Jena, is strongly recommended by the author quoted, and will doubtless be found an important aid in difficult investigations of the nature indicated. For ordinary work, however, a good achromatic condenser will furnish the necessary illumination, and it will be found that a good one-sixth or one-tenth inch objective answers very well for this purpose.

In order to render the number and distribution of the bacteria in an organ more evident, Koch recommends the following method. After staining with an aniline color, soak the sections in a weak solution of carbonate of potash, instead of acetic acid. By this means the animal tissues, including nuclei and plasma cells, lose their color, while the bacteria alone remain stained.

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[1] Traumatic Infective Diseases, English translation, p. 23. London, 1880.

Staining the Tubercle-Bacillus. — The following method was first recommended by Koch: One cubic centimetre of a concentrated alcoholic solution of methyl-blue is added to two hundred cubic centimetres of distilled water, and well shaken; then add, under continuous shaking, two tenths cubic centimetres of a ten per cent solution of caustic potash. The cover-glasses upon which tuberculous sputum has been spread and dried, or thin sections of a tuberculous lung, etc., are left in this solution for twenty-four hours. If the solution is heated in a water-bath at 40° C., the staining will be effected in much less time, — half an hour to an hour. The preparation is next treated with a concentrated aqueous solution of visuvin, which should be filtered just before it is used. After one or two minutes this is washed off with distilled water.

The visuvin solution discharges the blue color from the cells, nuclei and tissue elements generally, giving them a brown color, while the tubercle-bacilli retain their blue color and are readily recognized.

Baumgarten's Method. — In this method the sputum dried upon a cover-glass is moistened with a very dilute solution of potash, — one or two drops of a thirty-three per cent solution in a small watch-glass filled with distilled water. According to Baumgarten the bacilli may now be seen with a power of 400 to 500 diameters. The film of sputum is then allowed to dry, and the cover-glass is passed two or three times through the flame of an alcohol lamp, after which it is treated with an aqueous solution of one of the aniline colors. Baumgarten asserts that by this treatment the decomposition bacteria are deeply colored, while the tubercle-bacilli remain absolutely colorless.

Ehrlich's Method. — This method is considered by Koch a decided improvement upon his own, and has been employed with success by numerous observers in various parts of the world, especially for the examination of sputum. This is spread upon a cover-glass in as thin a layer as possible; and, in order to fix the albumen, the cover-glass is passed through the flame of a lamp three or four times, or kept at a temperature of 100 to 110° C. for an hour. The staining solution is prepared as follows: About five parts of pure aniline ("aniline oil") are added to one hundred parts of distilled water, well shaken, and filtered through a moistened filter. A saturated alcoholic solution of fuchsin, methyl-violet, or gentian-violet, is added to this mixture, drop by drop, until precipitation commences. The cover-glass is allowed to float upon this mixture, which may be conveniently prepared in a watch-glass, for fifteen minutes to half an hour; the side upon which the sputum has been spread is, of course, placed in contact with the staining fluid. The cover is then washed for a few seconds in a strong solution of nitric acid (one part of the commercial acid to two parts of distilled water). After this it must be thoroughly washed in pure water. By this process the stain is removed from everything but the tubercle bacilli, which retain the color imparted to them by the first operation. The ground-substance may now be stained so as to give a strong contrast with the bacilli; brown if the bacilli are violet, or blue if they have been stained red with fuchsin.

Gibbs' Method.—The following method of staining the tubercle-bacillus is recommended by Dr. Gibbs, of King's College, London:—

"The great advantage consists in doing away with the use of nitric acid. The stain is made as follows: Take of rosanilin hydrochloride two grammes, methyl blue one gramme; rub them up in a glass mortar. Then dissolve aniline oil 3 c. c. in rectified spirit 15 c. c.; add the spirit slowly to the stains until all is dissolved, then slowly add distilled water 15 c. c.; keep in a stoppered bottle. To use the stain: The sputum having been dried on the cover-glass in the usual manner, a few drops of the stain are poured into a test-tube and warmed; as soon as steam arises, pour into a watch-glass, and place the cover-glass on the stain. Allow it to remain for four or five minutes, then wash in methylated spirit until no more color comes away; drain thoroughly and dry, either in the air or over a spirit-lamp. Mount in Canada balsam. The whole process, after the sputum is dried, need not take more than six or seven minutes. This process is also valuable for sections of tissue containing bacilli, as they can be doubly stained without the least trouble. I have not tried to do this against time, but have merely placed the sections in the stain and allowed them to remain for some hours, and then transferred them to methylated spirit, where they have been left as long as the color came out. In this way beautiful specimens have been made, without the shrinking which always occurs in the nitric acid process." — *Lancet, May* 5, 1883.

Cheyne recommends the Weigert-Ehrlich staining solution. The formula is: of a filtered watery solution of aniline one hundred parts, of a saturated alcoholic solution of the basic aniline dye (methyl-violet, gentian-violet, fuchsin, etc.,) eleven parts; mix and filter. Rapid staining is obtained by warming the solution. The specimens are then decolorized by immersion in nitric acid (one part in two of water), and stained in a suitable contrast color. Very delicate sections are apt to be injured by immersion in the nitric acid. In this case, after staining them in the Weigert-Ehrlich fuchsin solution, they may be washed in distilled water, immersed in alcohol for a moment, and then placed in the following contrast stain for one or two hours: distilled water 100 c. c., saturated alcoholic solution of methyl blue 20 c. c., formic acid 10 minims.

According to Koch the bacillus of leprosy has the same color reaction as the tubercle-bacillus, while all other bacteria known to him differ from these in that the color imparted by one of the aniline dyes is discharged by visuvin and by nitric acid, used as above directed.

The tubercle-bacilli stained by any of the methods given are likely to fade after a time, especially when mounted in fluid, *e. g.*, glycerine or water.

§ 4. PHOTOGRAPHING BACTERIA. — Bacteria are prepared for photography as above directed; that is, a thin film of the material containing them is attached to a cover-glass by drying, stained, and mounted over a shallow cell containing fluid, or in balsam. For the larger forms methyl-violet is a suitable stain for this purpose; but a color less transparent for the actinic rays, such as aniline-brown or visuvin, will be required for the smaller species.

The writer has given an account of the technique of photo-micrography in another work, to which the reader desiring fuller information is referred.[1]

It is but fair to say that satisfactory results can only be obtained by the expenditure of a considerable amount of time and money, as the work must be done with high powers, and the technical difficulties to be overcome are by no means inconsiderable. The illustrations in the present volume may be taken as fair samples of what may be accomplished, and it will be found easier to criticise these than to improve upon them. Koch says, in his "Traumatic Infective Diseases": —

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[1] Photo-Micrographs and How to make them. James R. Osgood & Co., Boston, 1883.

> "In a former paper I expressed the wish that observers would photograph pathogenic bacteria, in order that representations of them might be as true to nature as possible. I thus felt bound to photograph the bacteria discovered in the animal tissues in traumatic infective diseases, and I have not spared trouble in the attempt. The smallest, and in fact the most interesting, bacteria, however, can only be made visible in animal tissues by staining them, and by thus gaining the advantage of color. But in this case the photographer has to deal with the same difficulties as are experienced in photographing colored objects, *e. g.*, colored tapestry. These have, as is well known, been overcome by the use of colored collodion. This led me to use the same method for photographing stained bacteria; and I have in fact succeeded, by the use of eosin-collodion, and by shutting off portions of the spectrum by colored glasses, in obtaining photographs of bacteria which had been stained with blue and red aniline dyes. Nevertheless, from the long exposure required and the unavoidable vibrations of the apparatus, the picture does not have sharpness of outline sufficient to enable it to be of use as a substitute for a drawing, or indeed even as evidence of what one sees. For the present, therefore, I must abstain from publishing photographic representations."

The difficulty of obtaining satisfactory photomicrographs of the smallest micro-organisms is illustrated in Figures 3 and 6, Plate XI. These represent the best results which the writer has been able to attain from a large number of trials in photographing the tubercle-bacillus. In Fig. 3 there are six of these bacilli, included within an epitheloid cell, from a specimen of the sputum of a tuberculous patient. The specimen is well stained with fuchsin by Ehrlich's method; and under the microscope the outlines of the cell, with its nucleus and the deeply-stained bacilli, are seen very distinctly. But in the attempt to photograph this object it was found to be impossible to bring all of the bacilli into focus at the same time; so that, while two bacilli are seen with tolerable distinctness, the others, being a little out of focus, can scarcely be distinguished. Fig. 6 represents the best result I have been able to obtain in photographing a single bacillus from the same source, stained in the same way, — with fuchsin. A close inspection will show that this bacillus is formed of a chain of four oval spores. When it is remembered that this is magnified 1,000 diameters, and has been stained and mounted *secundum artem*, it will not appear surprising that this minute bacillus escaped observation for so long a time; and upon comparing it with the *Anthrax bacillus* (Fig. 1 of the same plate), we see at once a very good reason for the discovery of the latter at a much earlier date.

§ 5. COLLECTION OF ATMOSPHERIC BACTERIA. — Fully developed bacteria are rarely found in the atmosphere; but we have ample evidence that the spores, or "germs," of numerous species are constantly present, in association with the reproductive elements of plants higher in the scale, and especially of the Mucorini and other microscopic fungi.

Considerable attention has been given to the study of atmospheric organisms with reference to the question of their possible connection with the epidemic prevalence of certain diseases. This is not a proper place to give a summary of the results attained; but the general statement may be made, that these have not been of a definite character, and that up to the present time no one has succeeded in demonstrating, in infected atmospheres, the presence of any specific forms of bacteria which were clearly connected with the deleterious effects produced in man or the lower animals by the respiration of such atmospheres. This line of investigation, however, has by no means been exhausted; and the careful and systematic study of atmospheric organisms in different localities, at different seasons, and under various circumstances as to sanitary conditions, is greatly to be desired. Any one who may be inclined to enter this field of investigation will do well to make himself familiar with what has already been done, and especially with the work of Maddox and Cunningham of England, and of Miquel of Paris. The last-named observer has given much time to the enumeration of atmospheric bacteria. He finds, as might have been expected, that they are more abundant during the summer months; and that they are less numerous immediately after a heavy rain, which has the effect of purifying the atmosphere, by washing out suspended particles.

Rain-water will always be found fertile in germs; and it is evident that when collected with care it represents the bacterial flora of the atmosphere at the time of its fall. We may therefore study this by means of culture-experiments, in which a variety of sterilized organic infusions are inoculated with one or more drops of rain-water which has just fallen. It is necessary to use many different culture-fluids, because various organisms require special media for their development.

Again, we may expose our sterilized organic infusions to the air, and thus permit them to become fertilized by the deposition of air-borne germs, the development of which is subsequently studied as they germinate upon the surface, or in the interior, of these infusions.

Solid culture-media are especially useful for this mode of investigation, and we may employ organic infusions to which three to five per cent of gelatine has been added, as recommended by Koch; and also a variety of cooked alimentary substances, such as moist bread, slices of boiled potato, turnip, onion, etc., various fruits (cooked or uncooked), meats of different kinds, etc. Upon the surface of these, if they are kept moist, and are placed in a culture-oven, maintained at a suitable temperature, little colonies of various organisms will form from the germination of spores deposited from the atmosphere. These will soon be recognized by the naked eye, and different species may often be distinguished by peculiarities as to growth, color, etc. It must be remembered that the *microbes* found in the atmosphere, so far as we now know, are accidentally present, and have originated elsewhere; *i. e.*, in decomposing material of organic origin from the surface of the earth. But, while we have no evidence that any known species finds the pabulum necessary for its development in the atmosphere, yet there is nothing improbable in the supposition that this may be true, and that there are species of bacteria which find in the atmosphere all of the conditions necessary for their rapid multiplication. We know that plants much higher in the scale, which are merely attached to others for support,—epiphytes,—derive their sustenance directly from the atmosphere; and it is easy to believe that, under exceptional circumstances as to the presence of organic matter and moisture, especially in tropical climates, or during the summer months in more northern latitudes, some of these minute microscopic plants may also multiply abundantly while suspended in the atmosphere.

To judge of the relative abundance of special forms of bacteria in the atmosphere, it will be necessary to resort to direct microscopic examination of the dust deposited upon exposed surfaces, or of the suspended particles collected by means of an aëroscope.

Various forms of aëroscope have been devised, the object of all being to cause a current of air to pass through a small aperture against a glass slide, the centre of which has been smeared with glycerine or some other viscid material, which serves to retain suspended particles. In the apparatus of Maddox, which was used by Cunningham in India, and a modification of which is employed by Miquel, a metal cone is made to face the wind by means of a weather-vane to which it is attached. A small aperture at the apex of the cone permits the concentrated current of air to project itself against a glass slide, smeared with glycerine, which is properly supported at a short distance back of this orifice. In the apparatus used by Klebs and Tomasi-Crudeli, in their investigations in the vicinity of Rome, a current of air is produced by a revolving "fan-wheel" moved by clock-work. The writer, in his investigations in Havana in 1879, and in New Orleans in 1880, used a water-aspirator, by means of which a measured quantity of air was caused to flow in a given time, through a small aperture, and to impinge upon a glass slide smeared with glycerine. Any one of these methods will answer the purpose; but the apparatus of Maddox seems to be the simplest, and has yielded very satisfactory results.

Instead of collecting the suspended organisms by means of a drop of glycerine attached to a glass slide, Pasteur has proposed to collect them by passing a current of air through a glass tube containing a loosely-packed filter of gun-cotton. This is subsequently dissolved in ether, and, upon evaporation of the ether, the particulate atmospheric impurities are found in the film of collodion remaining.

Examination of Water.—The bacterial flora of water from any source may be studied by the method already referred to in speaking of rain-water; viz., by using a small quantity to inoculate a variety of sterilized organic infusions, and observing the development of the various micro-organisms which make their appearance as the result of this procedure.

Dr. Angus Smith of Manchester has recently given a favorable account of results obtained by the gelatine method proposed by Koch. Pure fish-gelatine is added to the water to be tested, in sufficient quantity to form a gelatinous mass. If the water is pure, this remains for a long time unaltered; but, if impure from the presence of living organisms, the gelatine becomes liquefied in the vicinity of these, and little bubbles are formed, at the bottom of which the bacteria will be found.

§ 6. ATTENUATION OF VIRUS.—Various methods of producing physiological varieties of pathogenic bacteria, to be used in protective inoculations, have been proposed since Pasteur first announced (1880) that the microbe of fowl-cholera could be modified, by special treatment, in such a manner that it no longer produced a fatal form of the disease; and that fowls inoculated with this "attenuated virus" were subsequently protected against the disease, resisting inoculation with the most potent virus.

Method of Pasteur. — Pasteur found that the poison of fowl-cholera was most virulent when obtained from fowls which had died from a chronic form of the disease, and that this virus could be cultivated in chicken-bouillon for many successive generations without any diminution of its potency, if the interval between two successive inoculations was not greater than two months. But when a greater interval than this was allowed to elapse, the disease produced by inoculation was of a less serious character, and fewer deaths occurred. This diminution of virulence became more marked in proportion to the length of time during which a culture-solution containing the microbe remained exposed to the action of the atmosphere, and at last all virulence was lost, as a result of the death of the parasite. That this result is due to contact with the oxygen of the air is shown by the fact, that virus enclosed in sealed tubes does not undergo this modification, but retains its full virulence for many months. According to Pasteur, the various degrees of modification of virulence produced by prolonged exposure to oxygen are preserved by the cultivation, at short intervals, of the different grades of "attenuated virus."

Subsequent experiments with the virus of anthrax *(charbon)* gave similar results; and, under the direction of Pasteur, extensive protective inoculations have been practised in France with attenuated virus prepared by this method.

The time of exposure to oxygen is less for the anthrax bacillus than is required in the case of the micrococcus of fowl-cholera; and it is necessary to cultivate the bacillus in such a way as to prevent the development of spores, as these retain their virulence unchanged for many years. This is accomplished by cultivating the bacillus at a temperature of 42° to 43° C., at which point no spores are developed, the organism multiplying by fission only. Contact with the atmosphere for a month destroys entirely the vitality of the bacillus in such a culture, and in eight days it loses its deadly properties,—the temperature being maintained at the point mentioned. During this time the virus passes through successive degrees of attenuation. It is possible to restore the mitigated virus to its full activity by inoculating a guinea-pig one day old, which is killed by the operation, and using the blood of this animal to inoculate a second; and so on. After repeating this operation several times, the poison is said by Pasteur to regain its full vigor, and to be fatal to a sheep. In the same way the attenuated virus of fowl-cholera may be restored to full vigor by inoculating a small bird, — sparrow or canary, — to which it is fatal. After several successive inoculations from bird to bird, the virus resumes its original activity.

Method of Toussaint. — The effect produced upon pathogenic organisms by prolonged exposure to oxygen, Toussaint proposes to produce more expeditiously, by subjecting them for a short time to a temperature a little less than is required for the complete destruction of vitality.

According to Chauveau, this is best accomplished, in the case of *Bacillus anthracis*, by exposure for eighteen minutes to a temperature of 50° C. Exposure to this temperature for twenty minutes is said to kill the bacillus; while "heating for eighteen minutes produces an excellent attenuated virus for vaccination."

A first vaccination with feeble virus (heated to 50° for fifteen minutes), and a second inoculation, at the end of fifteen days, with a strong virus (blood heated to 50° for nine or ten minutes), preserves sheep from the effects of subsequent inoculations with virus of full strength. The heating must be in small tubes, not more than 1 mm. in diameter; and at the end of the time fixed these must be quickly withdrawn from the hot bath and plunged into cold water.

The blood of a guinea-pig which has just died from anthrax, at the end of thirty-six to forty-eight hours from the time of inoculation, is said to be a good active virus upon which to operate by this method. The attenuated virus, when used to inoculate a culture-fluid, develops more or less rapidly, according to the degree of attenuation. Bacilli heated for the longest time, and those subjected to the highest temperature, are the longest in showing signs of development.

Method of Chauveau. — Chauveau has attempted to test experimentally the question whether susceptible animals might not resist infection by a small number of active bacilli, and acquire immunity as the result of such inoculation. His results were favorable to the view that this is true as regards anthrax, at least; and Salmon has since adduced satisfactory evidence that it also applies to fowl-cholera. The method adopted by Chauveau consisted in diluting infected blood from the guinea-pig until a cubic centimetre of the mixture contains, as nearly as can be computed, the number of bacilli desired. A given quantity of this fluid was injected into the jugular vein of a sheep. Sheep of native French breeds were invariably killed when the number of bacilli introduced into the circulation was about one thousand. In an experiment in which two hundred and fifty bacilli were injected into each of five sheep, all withstood the dose, and four showed immunity when reinoculated at the end of six weeks. Immunity against symptomatic anthrax was also procured by the same procedure. Salmon, who has tested this method in fowl-cholera, has arrived at the following conclusions:—

"*First.*—A single disease-germ cannot produce this extremely virulent disease; it cannot even multiply sufficiently to produce the local irritation at the point of inoculation. When a quantity of virus was introduced into the tissues, which should have contained at least twelve germs, there was no effect, either general or local; but by increasing this one third, with the same birds, the local irritation appeared.

"*Second.*—It is apparent that the local resistance to the germs fails, while the constitutional resistance may still be perfect, and that in this case there may be a local multiplication of the organisms for two or three weeks without any disturbance of the general health.

"*Third.*—That this local multiplication of the virus is sufficient to grant a very complete immunity from the effects of such virus in the future."[1]

Method by Intravenous Injection.—In symptomatic anthrax, it has been found by Arloing, Cornevin, and Thomas, that intravenous injection of the virus produces in the calf, the sheep, and the goat only a slight indisposition, lasting for two or three days; and that subsequently the tumors characteristic of this disease are not developed as the result of inoculation in the muscles with the bacterium to which the disease is ascribed.

Attenuation of Virus by Chemical Reagents.—The attenuation of virulence which results from exposure to oxygen (method of Pasteur), or to an elevated temperature (method of Toussaint), seems to depend upon diminished reproductive activity of the pathogenic organism. Evidently the tissues of a susceptible animal are able to resist the invasion of a limited number of active germs (dilution of virus), and of a still greater number of those which are less active as a result of the treatment referred to.

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[1] The Med. Record, April 7, 1883, p. 371.

The writer has obtained evidence, in the course of his experiments relating to the comparative value of disinfectants, which goes to show that certain chemical reagents, also, may modify the virulence of pathogenic bacteria in a similar manner. In these experiments, the blood of a rabbit recently dead from induced septicæmia was the virulent fluid used as a test. The pathogenic organism in this case is a micrococcus, which is found in normal human saliva. In the published report of these experiments the following statement is made: —

> "The most important source of error, however, and one which must be kept in view in future experiments, is the fact that a protective influence has been shown to result from the injection of virus, the virulence of which has been modified, without being entirely destroyed, by the agent used as a disinfectant."[1]

Sodium hyposulphite and alcohol were the chemical reagents which produced the result noted in these experiments; but it seems probable that a variety of antiseptic substances will be found to be equally effective, when used in the proper proportion. Subsequent experiments have shown that neither of these agents is capable of destroying the vitality of the septic micrococcus in the proportion used (one per cent of sodium hyposulphite and one part of ninety-five per cent alcohol to three parts of virus), and that both have a restraining influence upon the development of this organism in culture-fluids.

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[1] Studies from the Biological Laboratory, Johns Hopkins University, Vol. II. No. 2, p. 205.

PART FOURTH.

GERMICIDES AND ANTISEPTICS.

A KNOWLEDGE of the vital resistance of the various species of bacteria to the action of different chemical reagents is important from several points of view. *First*, such information has an important bearing upon elementary biological problems, which are best studied in these simple unicellular plants; *second*, practical sanitation, and the preservation of various food-products, depend to a considerable extent upon the proper use of germicides and antiseptics; and, *third*, modern therapeutics has been largely influenced by the indications which this knowledge seems to furnish for the treatment of infectious diseases and surgical injuries.

By a *germicide* agent we mean one which has the power to destroy the vitality of the various species of bacteria known to us, including those disease-germs which have been demonstrated, such as the anthrax bacillus, the bacterium of symptomatic anthrax, the micrococcus of fowl-cholera, that of septicæmia in the rabbit, etc.

Germicides are also *antiseptics*, as the bacteria of putrefaction are killed by them as well as those mentioned. They may also arrest putrefactive decomposition in quantities less than are required to completely destroy putrefactive organisms. But an antiseptic is not necessarily a germicide; for experiment proves that certain substances arrest putrefaction which have not the power to kill the bacteria to which this is due. This they do by arresting the vital activity — multiplication — of the germs of putrefaction, or by so changing the nutritive pabulum required for the development of these germs that they are unable to appropriate it to their use.

If it were proven that the infectious character of every kind of infective material depended upon the presence of a specific living germ, as has been shown to be true in the case of certain kinds of infective material, *germicide* and *disinfectant* would be synonymous terms. Although this has not been proved, it is a significant fact that all of the disinfectants of established value have been shown by laboratory experiments to be potent germicides.

The antiseptic value of a substance is readily determined by a series of experiments in which it is added in various proportions to putrescible organic substances, and observing if, under favorable conditions as to temperature and moisture, putrefaction is arrested or prevented.

Some observers have made arrest of motion in the motile bacteria a test of germicide power. But it is evident that this is unreliable, and the only safe test is failure to multiply, under favorable conditions, in a suitable culture-fluid. This test requires care in its application, as contamination of the culture-fluid by other organisms than those which have been subjected to the action of the germicide agent would give a misleading result.

The method adopted by the writer in a series of experiments, the results of which are published in the "American Journal of the Medical Sciences," April, 1883, is very satisfactory and reliable. This consists in the use of the little culture-flasks, containing a sterilized organic infusion, prepared as directed on p. 179 of the present volume.

The bacteria which serve as a test are subjected to the action of the germicide in a small glass tube, previously sterilized by heat; and, after a given time, which in the experiments referred to was two hours, the fluid in the culture-flask is inoculated with a minute drop of fluid from the tube containing the test-organisms. The culture-flask is then placed in the oven, at a temperature of 98°–100° Fahr. At the end of twenty-four to forty-eight hours, inspection of the little flask will show in a very definite manner whether the germicide has been effectual or not: for the fluid will remain unchanged and transparent if the test-organisms were killed by the germicide agent; or, in case of failure, will have broken down, and will present an opalescent or milky appearance, from the abundant development which has taken place as the result of inoculation.

When a pathogenic organism is used to test the germicide power of chemical substances, we may inoculate living animals instead of sterilized culture-fluids. In this case, failure to produce the characteristic symptoms of the disease is, of course, to be taken as evidence that the vitality of the pathogenic germs was destroyed before inoculation. The most available organisms for such experiments, in the present state of science, are the bacillus of anthrax, the micrococcus of fowl-cholera, the bacterium of symptomatic anthrax, and the micrococcus of induced septicæmia in the rabbit.

In a series of experiments made by the writer in 1881, the last-named organism, as found in the blood of a rabbit recently dead, served as the test. The results were on the whole quite satisfactory and definite; but there are certain sources of error connected with this method which should be borne in mind. *First.* The test-organism may be modified as regards reproductive activity without being killed; and, in this case, a modified form of disease may result from the inoculation, of so mild a character as to escape observation. *Second.* An animal which has suffered this modified form of disease, enjoys protection, more or less perfect, from future attacks, and if used for a subsequent experiment may, by its immunity from the effects of the pathogenic test-organism, give rise to the mistaken assumption that this had been destroyed by the action of the germicide agent to which it had been subjected.

Vaccine virus has also been used by the writer, and by other experimenters, to test the comparative value of disinfectants. The method consists in dividing a certain quantity of virus from the same source into two parts, and subjecting one portion to the action of the agent to be tested, while the other is reserved to prove the reliability of the material used. A negative result from vaccination with the disinfected virus, and a positive result from that not disinfected, is evidence of the power of the disinfectant used to destroy the infective virulence of the material. The experiment must of course be made upon unvaccinated children, and it is best to make it in duplicate, two punctures being made upon one arm with the disinfected virus, and two in the other with that not disinfected.

A complete *résumé* of the experiments which have been made to determine the value of antiseptics and disinfectants would require more space than can be given to this subject in the present volume. Nor can the results obtained by different methods be brought together in tabular form; for discrepancies exist, due to various circumstances, and an extended discussion would be required to reconcile these, or to determine which were entitled to the greatest consideration. These discrepancies arise from the following circumstances: (*a*) The different bacteria which have been used as test-organisms differ within certain limits as regards vital resistance to the action of germicide agents. A like difference may occur in a particular species (*b*) as the result of the presence or absence of reproductive spores; (*c*) because of different conditions relating to the physical character of the material containing the germs; e. g., solid or fluid, coagulated masses, etc; (*d*) from a difference in the reaction of the media in which they are contained; (*e*) from a difference in the time of exposure to the action of the reagent.

The list which follows is arranged, for convenience, in alphabetical order. The writer has given his own results the precedence; and, as his experiments were made with special care by a method which offers the greatest possible security against error, he believes that they will be found, in the main, to be trustworthy. The letter S, enclosed in brackets, will be used to designate these; while results obtained from other sources will be followed by the name of the experimenter who has reported them.

In the author's experiments, unless otherwise stated in the text, the time of exposure to the action of the germicide agent was two hours. The septic micrococcus, frequently referred to below as one of the test-organisms employed, is from the blood of a rabbit recently dead, as the result of inoculation with human saliva; and, when "septicæmic blood" is spoken of, the blood of a rabbit which has fallen a victim to this form of septicæmia is meant. (Consult bibliography for titles of papers by the writer relating to this form of induced septicæmia in the rabbit.)

Acetic Acid. — This has the lowest preventive power in Group II. — the Organic Acids (Dougall).

Alcohol ranks low as a germicide, but is not without value as an antiseptic. Exposure to ninety-five per cent alcohol for forty-eight hours did not kill the bacteria in broken-down beef-tea (old stock). The septic micrococcus was destroyed by two hours' exposure to a twenty-four per cent solution. The micrococcus of gonorrhœal pus required a forty per cent solution (S).

"Pure or camphorated alcohol is largely used by surgeons in France to wash their instruments, but is evidently capable of giving only an illusory safety against morbid germs. . . . When saturated with camphor, alcohol does not destroy the virus of symptomatic anthrax" (Arloing, Cornevin, and Thomas). In the proportion of 1: 1.5, it destroys the bacteria which cause the acid fermentation of milk (Molke). 1: 1.18 destroys the bacteria of broken-down beef-tea, and 1: 20 prevents the development of these bacteria in sterilized beef-infusion (de la Croix). The micrococcus of pus multiplies freely in a culture-fluid containing five per cent of alcohol, but fails to multiply in a solution containing ten per cent. Exposure for half an hour to alcohol in the proportion of twelve per cent did not destroy the virulence of septic blood, which was injected into a rabbit with a fatal result. Twice this amount, however, proved effectual (S).

Aluminium Acetate.—The development of bacteria in pease-infusion is prevented by 1: 5,250 (Kühn). The development of bacteria in unboiled beef-infusion was prevented by 1: 6,310; and the bacteria of broken-down beef-tea were destroyed by 1: 478, while 1: 584 failed (de la Croix).

Aluminium Chloride. — "Group III. — Salts of the Alkaline Earths. Here chloride of aluminium is highest. . . . Were it not for the extremely high preventive point (1: 2,000) of this salt in the hay column, this group would occupy a comparatively subordinate position" (Dougall).

Ammonia does not destroy the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas); or the spores of the anthrax bacillus (Koch).

Aromatic Products of Decomposition.—Bauman first showed that phenol is developed in albuminous fluids during the process of putrefaction; and Salkowski found, in 1875, that old putrid fluids have antiseptic properties. Wernich has studied this subject, and finds that the aromatic products of decomposition,—skatol, phenyl, propionic acid, indol, kresol, phenyl acetic acid, and phenol,—arrest putrefaction, when present in organic infusions in small quantities, in the order named.

Arsenious Acid.—One per cent destroys spores of bacilli in ten days (Koch).

Benzoic Acid. — One part in 2,000 retards the development of spores (Koch). One part in 1,439 prevents development of bacteria in unboiled meat-infusion; 1: 2,010 does not. The bacteria of broken-down beef-tea are destroyed by 1: 77, while 1: 121 failed (de la Croix). In Group II. — the Organic Acids — benzoic has the highest preventive power (Dougall.)

Boric Acid in saturated aqueous solution (four per cent) failed to destroy the three test-organisms employed in the writer's experiments. But it prevented the development of the *M.* of pus in the proportion of 1: 200; of the *M.* of septicæmia in 1: 400, and of *B. termo* in 1 to 800. This difference, as regards ability to multiply in the presence of boric acid, accounts for the fact that micrococci have been observed to be present in the pus of wounds treated antiseptically with this substance, although no evidence of putrefaction could be discovered. A two per cent solution destroyed the virulence of septicæmic blood; but, in view of the fact that twice this amount did not kill the micrococcus to which this virulence is due, it is evident that the result obtained in inoculation experiments upon rabbits was due to the restraining — antiseptic — power of the reagent, and can not be taken as evidence of germicide power (S). The activity of fresh virus of symptomatic anthrax was destroyed by boric acid, one in five (twenty per cent) the time of exposure being forty-eight hours (Arloing, Cornevin, and Thomas). One part in 133 prevented the development of bacteria in tobacco-infusion, while 1: 200 failed (Bucholtz). One part in 58 prevented the development of bacteria in a vegetable infusion (peas), while 1: 81 failed; 1: 101 failed to preserve a solution of egg-albumen (Kühn). A five per cent solution was found by Koch to be inert, the test being the anthrax bacillus.

Bromine.—The spores of bacilli are killed by a two per cent aqueous solution of bromine. In the form of vapor this agent is superior, as regards rapidity of action, to chlorine and iodine (Koch). Bromine vapor is the most active agent for the destruction of the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). It destroys the ferment of sour milk (*Bacterium lactis*) in the proportion of 1: 348 (Molke). The bacteria of broken-down beef-tea are destroyed by 1: 336; and the development of bacteria in unboiled meat-infusion is prevented by 1: 5597 (de la Croix).

Camphor does not destroy the infective properties of vaccine except when it is exposed for at least a week in an air-chamber saturated with the volatile oil (Braidwood and Vacher). Alcohol saturated with camphor has no action upon the fresh virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). One part to 2,500 retards the development of anthrax spores (Koch).

Carbonic Acid.—Of five experimental vaccinations with lymph subjected to this gas, three succeeded (Braidwood and Vacher).

Carbonic Oxide.—Vaccine lymph may endure at least twenty-four hours' exposure to carbonic oxide without losing its specific properties (Braidwood and Vacher). This gas has no effect upon bacteria, which readily develop in it (Hamlet).

Carbolic Acid, in the proportion of one to two hundred, destroys *B. termo* and the septic micrococcus in active growth, while 1:25 failed to destroy the bacteria in broken-down beef-tea (old stock); the micrococcus of pus was destroyed by 1:225. The development of all of these organisms was prevented by the presence in a culture-fluid of 0.2 per cent = 1:500 (S). The micrococcus of swine plague multiplies abundantly in urine containing 1 per cent of carbolic acid, while the micrococcus of fowl-cholera is destroyed by six hours' exposure to a 1 per cent solution (Salmon). A 2 per cent solution destroys the bacterium of symptomatic anthrax (dried virus), the time of exposure being forty-eight hours (Arloing, Cornevin, and Thomas). The multiplication of bacteria in urine is not prevented by 1:100 (Haberkorn). In egg-albumen development of bacteria is prevented by 1:200 (Kühn). One part to 502 prevents the development of bacteria in unboiled meat-infusion; but the bacteria in broken-down beef-tea are not destroyed by a 10 per cent solution (de la Croix). A 5 per cent solution required two days to arrest the developing power of the spores of *Bacillus anthracis,* while a 1 per cent solution destroyed the bacilli themselves in two minutes. A solution of 1 : 850 prevented the multiplication of these bacilli in a suitable culture-medium. Carbolic acid in solution, in oil or in alcohol, is without effect upon the spores of *B. anthracis*, which germinated after being immersed 110 days and 70 days, respectively, in a 5 per cent solution in oil and in alcohol (Koch). The same author found that carbolic acid vapor, at 75° C., for two hours, failed to destroy anthrax spores. Chemical combinations with other substances were less efficacious than the pure acid. A 5 per cent solution of zinc sulpho-carbolate destroyed anthrax spores in five days; a 5 per cent solution of sodium phenate, in ten days, merely reduced their power of development, while sodium sulpho-carbolate failed to do this within the same time.

Chloral Hydrate failed to kill the micrococcus of pus in the proportion of 10 per cent, but was successful in the proportion of 20 per cent (S).

Chloroform.—A comparatively brief exposure to chloroform vapor entirely sterilizes vaccine lymph (Braidwood and Vacher). Chloroform has no effect upon the fresh virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). Chloroform is inert as regards the destruction of the spores of the anthrax bacillus (Koch). The development of bacteria in unboiled beef-infusion is prevented by 1 : 103; but 1 : 1.22 failed to destroy the bacteria of broken-down beef-tea (de la Croix).

Chlorine.—Exp. No. 37, Jan. 27, 1880.—Four children were vaccinated with virus from ivory points which had been exposed for six hours to an atmosphere containing one half per cent of chlorine (produced by the action of hydrochloric acid on the peroxide of manganese, and collected over warm water); also with four points, from the same lot, not disinfected. *Result:* Vaccination was unsuccessful in every case with the disinfected points, and successful with those not disinfected (S). Chlorine destroys the fresh virus of symptomatic anthrax, but is powerless against that which has been dried (Arloing, Cornevin, and Thomas). Chlorine is classed with bromine, iodine, and corrosive sublimate, as one of the most reliable agents for destroying the spores of anthrax (Koch). Development of bacteria in unboiled beef-infusion is prevented by the presence of one part in 15,606, and the bacteria of broken-down beef-tea are destroyed by 1 : 1,061 (de la Croix).

Chromic Acid, in the proportion of 1 : 1000, destroys the virulence of septicæmic blood (S). The development of anthrax spores is prevented by 1 : 5000; but chromic acid and its salts are inefficient for the destruction of these spores (Koch). Chromic acid was found to have a preventive power surpassing all others, its average being 1 : 2,200, while that of carbolic acid is only 1 : 226 (Dougall).

Citric Acid, in the proportion of 12 per cent, proved fatal to the micrococcus of pus, while 10 per cent failed (S).

Creosote, in the proportion of 1 : 200, is fatal to the micrococcus of pus (S).

Cupric Sulphate destroys the virulence of septicæmic blood in the proportion of 1 : 400 (S). The activity of dried virus of symptomatic anthrax is destroyed by a 20 per cent solution — time of exposure forty-eight hours (Arloing, Cornevin, and Thomas). The metallic salts, from their showing the highest average preventive power, form *Group I.* Sulphate of copper here has not only the highest individual average, but its three preventive points, in the three solutions, are very much higher than those of any other substance in the group (Dougall).

Ether does not destroy the spores of bacilli after thirty days' exposure (Koch). A brief exposure to the vapor of ether destroys the infective power of vaccine lymph (Braidwood and Vacher).

Eucalyptol retards the development of the spores of bacilli in the proportion of 1 : 2,500 (Koch). In the proportion of 1 : 205, the development of bacteria in unboiled meat-infusion is prevented. The bacteria in broken-down beef-tea are not destroyed by 1 : 14 (de la Croix).

Ferric Sulphate. — A saturated solution of this salt did not kill any of the test-organisms, and the use of this agent as a *disinfectant* would evidently be a serious error. It has, however, a decided value as an antiseptic, having prevented the development of all of the test-organisms in the proportion of 1 : 200. Although not fatal to the septic micrococcus in the proportion of 16 per cent, it prevents the development of septicæmia in the rabbit, after inoculation with septic blood to which it has been added, in the proportion of 1 : 400 (S). Exposure to a 20 per cent solution for forty-eight hours did not destroy the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas).

Ferri Chloridi Tinct. — A 4 per cent solution was fatal to the two species of *Micrococcus*, but failed to kill *B. termo.* The micrococci were not destroyed by a 2 per cent solution (S).

Glycerine, in the proportion of 12.5 per cent, destroyed the virulence of septicæmic blood, but failed at 10 per cent (S). Glycerine has no action upon the fresh virus of symptomatic anthrax (Arloing, Cornevin, and Thomas); and is inert as regards the spores of bacilli (Koch).

Heat. — The thermal death-point of the micrococcus of septicæmia (induced septicæmia in the rabbit) is 140° Fahr. (60° C.), the time of exposure being ten minutes; that of the micrococcus of gonorrhœal pus (believed to be identical with *M. ureae,* Cohn), is the same (S). The micrococcus of fowl-cholera is destroyed by exposure for fifteen minutes to a temperature of 132° Fahr. (Salmon). Nine or ten minutes' exposure to a temperature of 54° C. is sufficient to completely kill the bacilli in anthrax blood (Chauveau). Cohn has assigned 55° C. as the highest point at which bacteria studied by him have lived and developed. Van Tieghem says that this temperature is fatal to most of these organisms; but he has studied a bacillus which is able to multiply and form spores in a culture-fluid at a temperature as high as 74° C., but which ceased to multiply at 77°. Miquel had previously reported the existence, in the water of the Seine, of an immobile filamentous *Bacillus*, whch supports a temperature of 70° C., and which he has cultivated at this temperature in a neutral meat-infusion. This *Bacillus* was killed by a temperature of 71° to 72° C. The spores of *B. subtilis* resist for several hours a temperature of 100° C. (212° Fahr). The time required to kill these spores varies according to the nature of the liquid. In yeast-water, and in hay-infusion, they can resist a boiling temperature for five hours; while in distilled water they are killed after two or three hours. A temperature of 115° C. kills them very quickly (Chamberland). *Desiccated* septic blood does not lose its virulence at the end of forty days; or by being heated to 100° for from three to twenty-four hours, and the contained bacteria are capable of multiplication after such exposure (Lebedeff).

Hydrochloric Acid, in the proportion of 1 : 200, destroys the virulence of septicæmic blood (S). Hydrochloric acid gas destroys the contagion of vaccine (Braidwood and Vacher). A 2 per cent solution of muriatic acid kills the spores of the anthrax bacillus in ten days, while the development of these spores is prevented by 1 : 1,700 (Koch).

Hydrogen. — Bacteria may develop in an atmosphere of hydrogen (Hamlet).

Iodine (in aqueous solution with potassium iodide) destroys the septic micrococcus in the proportion of 1 : 1,000; the micrococcus of pus and *B. termo* in 1 : 500. It prevents the development of these organisms when present in a culture-solution in the proportion of 1 : 4,000 (S). The development of bacteria in tobacco-infusion is prevented by 1 : 5,714 (Bucholz); in boiled beef-infusion, 1 : 2,010; in unboiled, 1 : 10,020 (de la Croix). One part in 1,000 destroys the bacteria which produce the acid fermentation of milk (Molke); and 1 : 410 the bacteria of broken-down beef-tea (de la Croix).

Mercuric Bichloride. — All experimenters agree in placing this in the front rank as a germicide and antiseptic agent. One part in 40,000 prevents the development of the septic micrococcus, and but little less is required in the case of the micrococcus of gonorrhœal pus and of *B. termo.* To destroy the vitality of bacteria in broken-down beef-tea (old stock) required 1 : 10,000, while the above-mentioned micrococci were killed by 1 : 20,000 (S). The activity of the virus of symptomatic anthrax (dried virus) is destroyed by 1 : 5,000 (Arloing, Cornevin, and Thomas). The bacteria of broken-down beef-infusion are destroyed by 1 : 6,500, and their development in beef-tea prevented by 1 : 10,250 (de la Croix). One part in 20,000 prevents the development of bacteria in sterilized tobacco-infusion (Bucholz). One part in 1,000 destroys the spores of *Bacillus anthracis* in ten minutes (Koch).

Nitric Acid in the proportion of 1 : 400 destroys the virulence of septicæmic blood — time of contact, half an hour (S).

Nitrous Acid. — Exp. No. 36, Jan. 22, 1880. — Three children were vaccinated with ivory points which had been exposed for six hours to an atmosphere containing one per cent of nitrous acid gas (generated by pouring nitric acid on copper filings, and collected over mercury). *Result :* Vaccination was unsuccessful in each case, with disinfected points, and successful with the non-disinfected points from the same lot (S).

Oil of Mustard, in the proportion of 1 : 33,000, prevents the development of the spores of bacilli (Koch.) The development of bacteria in unboiled beef-tea is prevented by 1 : 3,353 ; and 1 : 40 destroys the vitality of bacteria in broken-down beef-tea (de la Croix).

Oil of Turpentine destroys the spores of bacilli in five days, and retards their development in the proportion of 1 : 75,000 (Koch). Turpentine has no action upon the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas).

Osmic Acid, in one per cent solution, destroys the spores of bacilli in one day (Koch).

Oxalic Acid, in saturated solution, destroyed the virulence of the fresh virus of symptomatic anthrax, but had no effect upon dried virus (Arloing, Cornevin, and Thomas).

Ozone impairs, and, if maintained long in contact, destroys the activity of vaccine lymph (Braidwood and Vacher). All germs suspended in the air, capable of developing in solutions of yeast from beer, are killed by ozone (Chappuis).

Oxygen. — The experiments of Pasteur upon the attenuation of virus show that long exposure to the oxygen of the atmosphere reduces the reproductive activity of the micrococcus of fowl-cholera and of the anthrax bacillus, and that after a time the vitality of these organisms is destroyed. The spores of the anthrax bacillus are, however, unaffected by prolonged exposure. Out of twelve experimental vaccinations with vaccine exposed to oxygen (time of exposure one to seven days), but one was successful, and in this case there is reason to believe that the exposure was imperfect (Braidwood and Vacher).

Picric Acid prevents the development of the spores of bacilli in the proportion of 1: 5,000 (Koch). The development of bacteria in beef-infusion is prevented by 1: 2,005, and the bacteria of broken-down beef-tea are destroyed by 1: 100 (de la Croix).

Potash. — Caustic potash in the proportion of two per cent was fatal to the micrococcus of septicæmia in one experiment, and failed in another; eight per cent failed to kill the micrococcus of pus, while ten per cent was successful; ten per cent failed to destroy the bacteria in broken-down beef-tea, and twenty per cent was successful (S). "Caustic potash has the minimum of preventive power, — 1 : 10. Such a mixture is highly caustic; still it was necessary to use it of such strength as at 1 : 25 vibriones and bacteria were abundant" (Dougall).

Potassium Arsenite (Fowler's solution of) failed to destroy the micrococcus of pus in the proportion of forty per cent. According to Koch, arsenite of potash prevents the development of anthrax spores in the proportion of 1 : 10,000.

Potassium Chlorate in the proportion of four per cent does not destroy the virulence of septicæmic blood (S). "Chlorate of potassium, so much used as a gargle in stomatitis, diphtheria, etc., where it is held to act by destroying certain fungi or germs of specific poison, has not only no preventive power, but actually accelerates decomposition" (Dougall).

Potassium Iodide gave no evidence of germicide power; exposure to the action of a saturated solution did not prevent the development of the bacteria of broken-down beef-tea (S).

Potassium Nitrate failed in 4 per cent solution to destroy the virulence of septicæmic blood (S).

Potassium Permanganate. — A 2 per cent solution destroys the virulence of septicæmic blood. The micrococcus of pus is destroyed by 1 : 800 (S). A 5 per cent solution destroys the fresh virus of symptomatic anthrax, but has no effect upon the dried virus (Arloing, Cornevin, and Thomas). One per cent will not destroy the spores of anthrax, but in the proportion of 1 : 3,000 their development is retarded (Koch). One part in three hundred prevents the development of bacteria in unboiled beef-infusion; and one part in thirty-five kills the bacteria of broken-down beef-tea (de la Croix).

Pyrogallic Acid. — A solution of one or two per cent prevents, for some months, the development of odors and of microscopic organisms; a solution of 2.5 per cent removes the odor from fluids in a state of putrefaction, and destroys bacteria (Bovet).

Pyroligneous Acid destroys the spores of the Anthrax bacillus in two days (Koch).

Quinine. — A 10 per cent solution of sulphate of quinine has no action upon the bacterium of symptomatic anthrax (Arloing, Cornevin, and Thomas). One per cent of quinine, *dissolved with muriatic acid*, destroys the spores of bacilli after ten days' exposure (Koch). The development of bacteria in a culture-fluid inoculated with a drop of turbid fluid from malarial soil is prevented by a solution of muriate of quinine of 1 : 900. From 1 : 1,000 to 1 : 1,500 non-putrid development begins. In a gelatine-culture from malarial soil no development occurred in solutions containing 1 : 1,500; non-putrid development occurred from 1 : 2,000 up to 1 : 3,000; and the development was accompanied by putrefaction when less than 1 : 9,000 was used (Ceri).

Salicylic Acid.—In the writer's experiments, this reagent was dissolved by means of sodium biborate, which, by itself, in saturated solution, has no germicide power. A two per cent solution was found to destroy the micrococcus of pus and *B. termo* in active growth; 4 per cent failed to destroy the bacteria in broken-down beef-tea (old stock). In the proportion of 1 : 200, this solution prevented the development of the micrococcus mentioned; in 1 : 800, that of *B. termo;* and the septic micrococcus in 1 : 400. But the antiseptic power exhibited by these figures does not differ from that obtained by the use of the solvent employed when used alone. The virus of symptomatic anthrax is destroyed by forty-eight hours' exposure to a solution of salicylic acid of 1 : 1,000, and by saturated salicylic alcohol (Arloing, Cornevin, and Thomas). Salicylic acid dissolved in oil and in alcohol, in 5 per cent solution, does not destroy the spores of the anthrax bacillus (Koch). 1 : 200 destroys the bacteria of sour milk (Molke). 1 : 343 killed the bacteria of beef-tea, and 1 : 1,121 prevented the development of bacteria in unboiled meat-infusion exposed to the air (de la Croix). The bacteria of tobacco-infusion were destroyed by 1 : 362, and their multiplication prevented by 1 : 932 (Bucholz). 1 : 724 prevented the development of bacteria in a vegetable infusion, and 1 : 1,000 in a solution of egg-albumen (Kühn).

Soda. — Caustic soda destroys the virulence of septicæmic blood in the proportion of 1 : 400 (S). A one-in-five solution of soda destroys the virus of symptomatic anthrax when fresh, but has no effect upon dried virus (Arloing, Cornevin, and Thomas).

Sodium Biborate. — The results obtained with this salt correspond, in the writer's experiments, with those given by boric acid. The virulence of septic blood, as shown by inoculation of rabbits, was destroyed by 2.5 per cent, while 1.25 per cent failed. That this is not due to germicide power is shown by the fact that a saturated solution does not kill the septic micrococcus, as proved by culture-experiments. It also failed with *B. termo* and the *M.* of pus. The multiplication of all of these organisms was, however, prevented by the presence of 1 : 200 in a culture-fluid, and *B. termo* failed to multiply in the presence of 1 : 400 (S). A 20 per cent solution does not destroy the virulence of the virus of symptomatic anthrax, as proved by inoculation experiments (Arloing, Cornevin, and Thomas). In the proportion of 1 : 107, the development of bacteria in unboiled beef-infusion is prevented, while 1 : 161 failed. 1 : 12 failed to kill the bacteria in broken down beef-tea (de la Croix).

Sodium Chloride, in 5 per cent solution, failed to destroy the virulence of septicæmic blood (S). Common salt ranks low as a preventive (Dougall). It is well known that meat may become putrid in a weak solution of brine, but the extended use of salt as a preservative agent demonstrates its antiseptic power, when used in a sufficiently strong solution. It is doubtful, however, whether infectious disease germs (spores) would be destroyed by the most concentrated solution. "A saturated solution of cloride of sodium did not destroy the virus of symptomatic anthrax in forty-eight hours' contact" (Arloing, Cornevin, and Thomas).

Sodium Hyposulphite. — This salt, in the writer's experiments, gave no evidence whatever of germicide power. In saturated solution it failed to kill the bacteria in broken-down beef-tea, and the *M.* of pus was not destroyed by exposure for two hours to a thirty-two per cent solution. Nor was the development of the last-named organism prevented by the presence of this salt in a culture-solution in the proportion of eight per cent (S). Exposure for forty-eight hours to a fifty per cent solution does not destroy the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). Chloride of lime, hard soap, chloralum, and common salt are low preventives. The *hyposulphite*, borate, and sulphate of soda are useless as such (Dougall).

Sodium Sulphite. — The results obtained correspond with those reported in the case of sodium hyposulphite (S.)

Sodium Salicylate failed to destroy any of the test-organisms used in the writer's experiments, in the proportion of four per cent. But the virulence of septicæmic blood was destroyed by 1.25 per cent; it must therefore have a restraining influence upon the development of the septic micrococcus, and doubtless upon other forms of bacteria also.

Sulphuric Acid destroys *B. termo* and the two species of micrococcus experimented upon in the proportion of 1 : 200 ; but a four per cent solution failed to destroy the bacteria in broken-down beef-tea (old stock), doubtless because of the presence of reproductive spores. The multiplication of the bacteria mentioned was prevented by the presence of this acid in a culture-solution, in the proportion of 1 : 800 (S). One part in 3,353 prevented the development of bacteria in unboiled meat-infusion, and 1 : 72 destroyed the bacteria of broken-down beef-tea (de la Croix). One part in 161 destroyed bacteria developed in tobacco-infusion (Bucholz).

Sulphurous Acid. — Exp. No. 35, Jan. 15, 1880. — Five children were vaccinated from ivory points which had been exposed for six hours to an atmosphere (dry) containing one per cent of sulphur dioxide (collected over mercury), and with five other points, from the same lot, not disinfected. *Result:* Vaccination was unsuccessful in each case with the disinfected points, and successful with those not disinfected (S). In four experiments in which dry vaccine was exposed to the fumes of sulphurous acid, for ten minutes, its infecting power was destroyed (Baxter). Sulphurous acid has no influence upon the bacteria of symptomatic anthrax (Arloing, Cornevin, and Thomas). It is powerless against the spores of the anthrax bacillus (Koch). In the proportion of 1 : 12,649, the development of bacteria in uncooked beef-infusion is prevented, and in 1 : 135 it destroys the vitality of the bacteria of broken-down beef-tea (de la Croix).

Sulphuretted Hydrogen. — Bacteria develop readily in the presence of sulphuretted hydrogen (Hamlet).

Tannic Acid, in the proportion of one per cent, destroys the virulence of septic blood (S). A one-in-five solution of tannic acid has no effect upon the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). A five per cent solution does not kill the spores of anthrax (Koch).

Thymol dissolved in alcohol destroys the virulence of septicæmic blood (time of exposure half an hour), in the proportion of 1 : 400 (S). Thymol retards the development of anthrax spores in the proportion of 1 : 80,000 (Koch). One part in 200 kills the bacteria of tobacco-infusion (Bucholz); one part in 50 the sour-milk ferment (Molke); and one in 20 the bacteria of broken-down beef-tea (de la Croix). The development of bacteria in unboiled beef-infusion is prevented by 1 : 1,340 (de la Croix), and in Pasteur's fluid by 1 : 2,000 (Bucholz).

Zinc Chloride destroys the micrococcus of gonorrhœal pus in the proportion of two per cent; the septic micrococcus failed to multiply after exposure to one part in 200 (S). A five per cent solution failed within a month to weaken the developing power of splenic fever spores (Koch). Liquor zinci chloridi (Squibbs) failed to kill the micrococcus of pus, in the proportion of eight per cent (S).

Zinc Sulphate, in the proportion of twenty per cent, does not kill the micrococcus of pus; but in the proportion of 1.25 per cent, it destroys the virulence of septicæmic blood. This is no doubt due to restraining power, and cannot be taken as evidence that the vitality of the septic micrococcus was destroyed (S).

PART FIFTH.

BACTERIA IN INFECTIOUS DISEASES.

No more important question has ever engaged the attention of physicians, of sanitarians, or of biologists, than that which relates to the *rôle* of the bacteria in infectious diseases. The practical results of etiological studies, so far as the prevention and cure of disease are concerned, are likely to be much greater than those which have been gained by the pathologists; and, if the time ever comes, as now seems not improbable, when we can say with confidence, infectious diseases are parasitic diseases, medicine will have established itself upon a scientific foundation. But this generalization, which some physicians think is justified, even now, by the experimental evidence which has been so rapidly accumulating during the past decade, would, in the opinion of the writer, be premature in the present state of science. And, for the present, it seems wiser to encourage additional researches rather than to attempt to generalize from the data at hand. For much of the evidence offered in favor of this view is open to question;

and even where we do not doubt the scientific accuracy of an observer, we may differ from him as to the interpretation of the facts which he has recorded. Those who have had the most experience in this difficult field of investigation, are commonly the most critical and exacting with reference to the alleged discoveries of others. And it is now generally admitted that the only satisfactory proof that a certain micro-organism bears a causal relation to a disease with which it is associated is that which is obtained by a series of culture experiments, in which the organism is completely isolated from the non-living constituents of the infective material containing it, and in the production of the disease in question by inoculation experiments with such a "pure-culture." The unimpeachable nature of this proof, when the experiment is properly made and frequently repeated with the same result, is made apparent in the following quotation from a paper by the writer relating to "a fatal form of septicæmia in the rabbit."[1]

"In my previous paper I related a series of experiments commenced July 6th, to which I must refer the reader as properly introducing the following: —

"The culture-fluid (No. 6) used in *Experiment No.* 3 (July 26th) was laid aside in an hermetically-sealed culture-flask until September 12th, when a minute drop was used to inoculate sterilized *bouillon* in culture-tube No. 7. This, placed in a culture-oven at 100° Fahr. for twenty-four hours, became clouded, and upon microscopical examination proved to be pervaded with the identical micrococcus heretofore described and photographed (See Fig. 2, Plate IX.). A drop of culture No. 7 was in like manner used to inoculate culture No. 8, and the next day, this being also pervaded by the micrococcus, was used in the following experiment: —

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[1] Med. Times, Phila., Nov. 4th, 1882, p. 81.

"*Exp. No.* 4. — September 14th. — Injected ten minims of culture No. 8 into a full-grown rabbit. *Result:* This animal died at 9 A. M. September 15th, and a microscopical examination made at once demonstrated the presence of the micrococcus in great numbers in the blood and in effused serum in the sub-cutaneous connective tissue.

"*Remarks.* — This experiment shows that the micrococcus retained its vitality and its full virulence at the end of six weeks, and, very conclusively, that the virulence of the culture-fluid is due to the presence of the micrococcus, and not to a hypothetical chemical virus found in the first instance in human saliva and subsequently in the blood of a rabbit inoculated with this fluid. For the benefit of those who have not calculated the degree of dilution which such a hypothetical chemical virus would undergo in such a series of culture experiments, I submit the following simple calculation: —

"My culture-tubes contain about a fluidrachm of sterilized *bouillon.* The amount of blood introduced into culture No. 1, as seed, was considerably less than a minim; but for convenience I will suppose that one minim is used each time to start a new culture, — that is, the original material is diluted 60 times in the first culture, 3,600 times in the second, 216,000 times in the third, and in the eighth culture it will be present in the proportion of one part in 1,679,611,600,000,000. Yet a few minims of this eighth culture possesses all the virulence of the first.

"Look at it from another point of view. The few minims of culture-fluid introduced beneath the skin of a rabbit contain a micrococcus presenting definite morphological characters. The blood of the animal which falls a victim to experimental inoculation with this fluid is filled within forty-eight hours with the same micro-organism in numbers far exceeding the normal histological elements, — red and white corpuscles; yet some very conservative physicians still claim that the invading parasite is without import, a mere epi-phenomenon, while the infinitesimal portion of a hypothetical chemical virus is credited with this malignant potency."

When, in addition to this, we remember that potent chemical poisons, especially when injected subcutaneously, act promptly, and that their poisonous effect bears a relation to the dose in which they are administered, whereas a rabbit subjected to an experimental inoculation with septic blood, or with a culture-fluid remotely inoculated with this material, shows no signs of ill-health for many hours, — eighteen hours or more, — and that it is only when sufficient time has elapsed to permit of the abundant development of the micrococcus that serious symptoms are developed, we shall see that but one conclusion can be drawn as regards the *rôle* of the micrococcus.

It is by experimental evidence of this nature that Koch, Pasteur, and many others have demonstrated beyond question that the disease known as anthrax is produced by a parasitic micro-organism, — the *Bacillus anthracis*; that the last-named investigator has established the etiological *rôle* of the micrococcus of fowl-cholera; and that Koch has proved that a form of induced septicæmia in mice, which he has especially studied, is due to a minute bacillus.

It has been suggested that the parasitic micro-organism in these diseases is, perhaps, only a secondary cause, being merely a carrier of the non-living ferment, which is the special poison of the disease. This hypothesis, also, is excluded by inoculation experiments with a pure-culture, sufficiently removed from the natural infective material. For the organisms introduced into culture No. 1, as seed, disappear as quickly from successive cultures as does the non-living material with which they are associated, and we may very soon leave them out of the account, although each successive culture-fluid is invaded throughout by their numerous progeny.

Having determined for a certain infectious disease that its transmissibility depends upon the presence in the infective material of a living micro-organism, the question naturally arises as to the *modus operandi* of this parasite. Does it produce death by appropriating something from the vital fluid, or from the tissues invaded by it, — e. g., oxygen, which is essential for the maintenance of vital processes in the living animal? Or does it, at the same time that it appropriates material for its own nutrition, evolve some poisonous chemical product which is the immediate cause of the morbid phenomena in the infected animal? Or does it produce death by the mechanical effects which result from its presence in such vast numbers, i. e., by blocking up the capillaries and the formation of emboli ?

There can be little doubt that, in these acute infectious diseases, the parasite injures its host in all three of the ways indicated, and that a fatal result is to be ascribed to the three causes mentioned conjointly.

A most difficult and important question in connection with these diseases is that which relates to the rationale of the immunity produced by protective inoculations practised by one of the methods described in PART FOURTH of the present volume. In these protective vaccinations, the virus used is either greatly diluted or is modified as regards the reproductive activity of the parasite by exposure to oxygen, by heat, or by certain chemical reagents. A susceptible animal, when inoculated with virus "attenuated" by one of these methods, does not succumb to the attacks of the parasite, but, after experiencing a mild form of the disease, recovers, and is subsequently protected from the effects of full doses of unmodified virus.

This recovery after inoculation with attenuated virus is more easy to understand than is the subsequent protection. There is evidently some provision of nature by which invading organisms may be disposed of when they do not multiply too quickly, but which fails when they have very great reproductive activity, and when the conditions within the living animal are extremely favorable to their development. These conditions doubtless relate mainly to the composition and temperature of the culture-medium — i. e., of the blood of the animal — and consequently vary in different species of animals. But that the composition of the blood should be changed materially and permanently in the same animal, as a result of the mild form of disease which follows protective inoculation, it is difficult to believe. Yet this is the explanation given by Pasteur of the immunity afforded by such inoculations. This change is supposed to consist in the removal of some material essential for the nutrition of the microbe, which is exhausted during the attack, and never reproduced. This view is sustained in the following language: —

> "It is the life of a parasite in the interior of the body which produces the malady commonly called '*cholera des poules*,' and which causes death. From the moment when this culture (*i. e.*, the multiplication of the parasite) is no longer possible in the fowl, the sickness cannot appear. The fowls are then in the constitutional state of fowls not subject to be attacked by the disease. These last are as if vaccinated from birth for this malady, because the fœtal evolution has not introduced into their bodies the material necessary to support the life of the microbe; or these nutritive materials have disappeared at an early age.
>
> "Certainly one should not be surprised that there may be constitutions sometimes susceptible and sometimes rebellious to inoculation — that is to say, to the cultivation of a certain virus, when, as I have an-

nounced in my first note, one sees a preparation of beer yeast made exactly like one from the muscles of fowls (*bouillon*) to show itself absolutely unsuited for the cultivation of the parasite of fowl cholera, while it is admirably adapted to the cultivation of a multitude of microscopic species, notably to the *bactéride charbonneuse* (*Bacillus anthracis*).

"The explanation to which these facts conduct us, as well of the constitutional resistance of some individuals, as of the immunity produced by protective inoculations, is only natural when we consider that every culture, in general, modifies the medium in which it is effected; a modification of the soil when it relates to ordinary plants; a modification of plants and animals when it relates to their parasites; a modification of our culture liquids when it relates to *mucédines*, *vibrioniens*, or ferments.

"These modifications are manifested and characterized by the circumstance that new cultivations of the same species in these media become promptly difficult or impossible. If we sow *chicken-bouillon* with the microbe of fowl-cholera, and, after three or four days, filter the liquid in order to remove all trace of the microbe, and subsequently sow anew in the filtered liquid this parasite, it will be found quite powerless to resume the most feeble development. The liquid, which is perfectly limpid after being filtered, retains its limpidity indefinitely.

"How can we fail to believe that by cultivation in the fowl of the attenuated virus, we place its body in the state of this filtered liquid, which can no longer cultivate the microbe? The comparison can be pushed still further; for, if we filter the *bouillon* containing the microbe in full development, not on the fourth day of culture, but on the second, the filtered liquid will still be able to support the development of the microbe, although with less energy than at the outset. We comprehend, then, that after a cultivation of the modified (*attenué*) microbe in the body of the fowl, we may not have removed from all parts of its body the aliment of the microbe. That which remains will permit, then, a new culture, but in a more restricted measure.

"This is the effect of a first inoculation; subsequent inoculations will remove progressively all the material necessary for the development of the parasite.

"Is this the only possible explanation of the phenomenon? No; we may admit the possibility that the development of the microbe, in place of removing or destroying certain matters in the bodies of the fowls, adds, on the contrary, something which is an obstacle to the future development of this microbe. The history of the life of inferior beings authorizes such a supposition. The excretions resulting from vital processes may arrest vital processes of the same nature. In certain fermentations we see antiseptic products make their appearance during, and as a result of, the fermentation, which put an end to the active life of the ferments, and arrest the fermentations long before they are completed. In the cultivation of our microbe, products may have been formed the presence of which, possibly, may explain the protection following inoculation.

"Our artificial cultures permit us to test the truth of this hypothesis. Let us prepare an artificial culture of the microbe, and after having evaporated it, *in vacuo*, without heat, let us bring it back to its original volume by means of fresh chicken *bouillon*. If the extract contains a poison for the life of the microbe, and if this is the cause of its failure to multiply in the filtered liquid, the new liquid should remain sterile. Now this is not the case. We cannot, then, believe that during the life of the parasite certain substances are produced which are capable of arresting its ulterior development." — (*Comptes rendus Acad. des Sc.*, XC. pp. 952–958.)

It is a little surprising that after disproving, by the experimental method, the hypothesis last mentioned, which had been proposed by a member of the French Academy in explanation of the phenomenon in question, Pasteur did not, in accordance with his usual custom, attempt to establish his own hypothesis upon a firm foundation by an experiment which at once suggests itself. If a fowl which is protected against cholera, or an animal which is protected against anthrax, owes this protection to the fact that a certain material which is required for the development of the microbe of fowl-cholera, or for the anthrax bacillus, has been exhausted in the course of the modified form of the disease to which immunity is due, then the flesh of such an animal, made into *bouillon*, should not constitute a proper culture-medium for the organisms in question. The writer ventures to predict that the result of such an experiment would not be favorable to Pasteur's hypothesis, and that it will be found that the micrococcus of fowl-cholera can be cultivated in *bouillon* made from the flesh of a protected animal, and that the bacillus of anthrax may multiply freely in the blood, or in an infusion of the flesh, of an animal which, before it was killed for the experiment, possessed immunity against the disease anthrax. The writer long since proposed to himself to make the experiment, but has not yet been able to do so. The matter is mentioned here in the hope that some one more favorably situated for pursuing experimental work will consider it of sufficient importance to induce him to test it in the manner indicated. In the meantime I take the liberty of quoting, from a paper published in 1881, certain extracts in which my reasons are given for doubting the correctness of the hypothesis of Pasteur, and in which another explanation is offered: —

> "Let us see where this hypothesis leads us. In the first place, we must have a material of small-pox, and a material of measles, and a material of scarlet fever, etc., etc. Then we must admit that each of these different materials has been formed in the system and stored up for these emergencies, — attacks of the diseases in question, — for we can scarcely conceive that they were all packed away in the germ-cell of the mother and the sperm-cell of the father of each susceptible individual. If, then, these peculiar materials have been formed and stored up during the development of the individual, how are we to account for the fact that no new production takes place after an attack of any one of the diseases in question?
>
> "Again, how shall we account for the fact that the amount of material which would nourish the small-pox germ, to the extent of producing a case of confluent small-pox may be exhausted by the action of the attenuated virus (germ) introduced by vaccination? Pasteur's comparison of a fowl protected by inoculation with the microbe of fowl-cholera, with a culture-fluid in

which the growth of a particular organism has exhausted the pabulum necessary for the development of additional organisms of the same kind, does not seem to me to be a just one, as in the latter case we have a limited supply of nutriment, while in the former we have new supplies constantly provided of the material—food—from which the whole body, including the hypothetical substance essential to the development of the disease-germ, was built up prior to the attack. Besides this, we have a constant provision for the elimination of effete and useless products.

"This hypothesis, then, requires the formation in the human body, and the retention up to a certain time, of a variety of materials, which, so far as we can see, serve no purpose except to nourish the germs of various specific diseases, and which, having served this purpose, are not again formed in the same system, subjected to similar external conditions, and supplied with the same kind of nutriment.

"The difficulties into which this hypothesis leads certainly justify us in looking further for an explanation of the phenomenon in question. This explanation is, I believe, to be found in the peculiar properties of the protoplasm, which is the essential frame-work of every living organism. The properties referred to are: the tolerance which living protoplasm may acquire to certain agents which, in the first instance, have an injurious or even fatal influence upon its vital activity, and the property which it possesses of transmitting its peculiar qualities, inherent or acquired, through numerous generations, to its offshoots or progeny.

"Protoplasm is the essential living portion of the cellular elements of animal and vegetable tissues; but as our microscopical analysis of the tissues has not gone beyond the cells of which they are composed, and is not likely to reveal to us the complicated molecular structure of the protoplasm upon which, possibly, the properties under consideration depend, it will be best, for the present, to limit ourselves to a consideration of the living cells of the body. These cells are the direct descendants of pre-existing cells, and may all be traced back to the sperm-cell and germ-cell of the parents. Now, the view which I am endeavoring to elucidate is, that during a non-fatal attack of one of the specific diseases, the cellular elements implicated, which do not succumb to the destructive influence of the poison, acquire a tolerance to this poison which is transmissible to their progeny, and which is the reason of the exemption which the individual enjoys from future attacks of the same disease.

"The known facts in regard to the hereditary transmission, by cells, of acquired properties, make it easy to believe in the transmission of such a tolerance as we imagine to be acquired during the attack; and if it is shown by analogy that there is nothing improbable in the hypothesis that such a tolerance is acquired, we shall have a rational explanation, not of heredity and the mysterious properties of protoplasm, but of the particular result under consideration. The transmission of acquired properties is shown in the budding and grafting of choice fruits and flowers, produced by cultivation, upon the wild stock from which they originated. The acquired properties are transmitted indefinitely; and the same sap which on one twig nourishes a sour crab-apple, on another one of the same branch is elaborated into a delicious pippin. . .

"The tolerance to narcotics — opium and tobacco, and to corrosive poisons — arsenic, which results from a gradual increase of dose, may be cited as an example of acquired tolerance by living protoplasm to poisons, which at the outset would have been fatal in much smaller doses.

"The immunity which an individual enjoys from any particular disease must be looked upon as a power of resistance possessed by the cellular elements of those tissues of his body which would yield to the influence of the poison in the case of an unprotected person. . . . The resistance of living matter to certain destructive influences is a property depending upon vitality. Thus, living protoplasm resists the action of the bacteria of putrefaction, while dead protoplasm quickly undergoes putrefactive changes." — *Am. J. of the Med. Sciences, April*, 1881, *p.* 375.

The hypothesis of Pasteur would account for the fact that one individual suffers a severe attack and another a mild attack of an infectious disease, after being subjected to the influence of the poison under identical circumstances, by the supposition that the pabulum required for the development of this particular poison is more abundant in the body of one individual than in the other. The explanation which seems to us more satisfactory, is that the vital resistance offered by the cellular elements in the bodies of these two individuals was not the same for this poison. It is well known that in conditions of lowered vitality, resulting from starvation, profuse discharges, or any other cause, the power to resist disease-poisons is greatly diminished, and, consequently, that the susceptibility of the same individual differs at different times.

From our point of view, the blood, as it is found within the vessels of a living animal, is not simply a culture-fluid maintained at a fixed temperature; but, under these circumstances, is a tissue, the histological elements of which present a certain vital resistance to pathogenic organisms which may be introduced into the circulation.

If we add a small quantity of a culture-fluid containing the bacteria of putrefaction to the blood of an animal, withdrawn from the circulation into a proper receptacle, and maintained in a culture-oven at blood-heat, we will find that these bacteria multiply abundantly, and evidence of putrefactive decomposition will soon be perceived. But, if we inject a like quantity of the culture-fluid with its contained bacteria into the circulation of a living animal, not only does no increase and no putrefactive change occur, but the bacteria introduced quickly disappear, and at the end of an hour or two the most careful microscopical examination will not reveal the presence of a single bacterium. This difference we ascribe to the vital properties of the fluid as contained in the vessels of a living animal; and it seems probable that the little masses of protoplasm known as white blood corpuscles are the essential histological elements of the fluid, so far as any manifestation of vitality is concerned.

The writer has elsewhere suggested that the disappearance of the bacteria from the circulation, in the experiment above referred to, may be effected by the white corpuscles, which, it is well known, pick up, after the manner of amœbæ, any particles, organic or inorganic, which come in their way. And it requires no great stretch of credulity to believe that they may, like an amœba, digest and assimilate the protoplasm of the captured bacterium, thus putting an end to the possibility of its doing any harm.

In the case of a pathogenic organism we may imagine that, when captured in this way, it may share a like fate if the captor is not paralyzed by some potent poison evolved by it, or overwhelmed by its superior vigor and rapid multiplication. In the latter event, the active career of our conservative white corpuscle would be quickly terminated, and its protoplasm would serve as food for the enemy. It is evident that in a contest of this kind the balance of power would depend upon circumstances relating to the *inherited* vital characteristics of the invading parasite and of the invaded leucocyte.

That different pathogenic organisms of the same species may differ as to their power to overcome the vital resistance of living animals is amply proved by experiment. We have examples of this in the attenuated virus of anthrax and of fowl-cholera. These physiological varieties, as Pasteur calls them, may be produced at will by one of the methods heretofore referred to. They differ from the unmodified virus in vital activity, and this is especially manifested in their diminished reproductive power.

In the great laboratory of nature, like causes must produce similar results; and there can be little doubt that physiological varieties, or breeds, of the different species of bacteria are constantly being produced and destroyed by the operation of natural causes. Under the influence of a favorable temperature and of abundant pabulum, these minute plants multiply abundantly; and, in accordance with the laws of natural selection, there must be a constant tendency among them to develop those characters which are most favorable to their preservation, — e. g., a capacity for rapid multiplication, and to adapt themselves to their environment.

If we suppose that under certain circumstances the conditions relating to environment approach those which would be found within the body of a living animal, we can easily understand how a micro-organism, which has adapted itself to these conditions, may become a pathogenic organism, when by any chance it is introduced into the circulation of such an animal. The culture-fluid — blood — and temperature being favorable, it is only a question of superiority by vital resistance on the one hand, or by reproductive activity on the other.

That harmless species of bacteria may develop pathogenic properties in the manner indicated, seems extremely probable; and we should *a priori* expect that such a result would occur more frequently in the tropics, where the elevated temperature and abundance of organic pabulum furnish the favorable conditions required. In this way we may, perhaps, explain the origin of epidemics of pestilential diseases, such as yellow fever and cholera. If these diseases do not, at the present day, originate in the manner indicated, they at all events have their permanent abiding place in tropical countries. Although the specific germs of these diseases have not been demonstrated, there is strong reason for believing that they result from the direct or indirect action of living ferments. For there is abundant evidence to prove that the specific poisons to which they are due *may multiply indefinitely external to the bodies of the sick.* Such multiplication is a property of living matter only. Moreover, the conditions which favor this multiplication — an elevated temperature and the presence of decomposing organic material — are exactly the conditions required for the development of low organisms.

The experimental transformation of the harmless hay-bacillus (*B. subtilis*) into the deadly *Bacillus anthracis* has been claimed by Buchner and by Nägeli; and Prof. Greenfield claims to have transformed, by a series of culture experiments, the anthrax bacillus into a harmless form not distinguishable from the hay bacillus. Koch insists, however, that these are distinct species, and the weight of evidence seems to be in favor of this view. However this may be, it is beyond question that the anthrax bacillus may undergo a remarkable modification as regards virulence; and

Pasteur asserts that this virulence may be restored by inoculating guinea-pigs but a day old, which succumb to this attenuated virus, although those which are five or six days old are proof agains it. After several successive inoculations, older guinea-pigs are killed, and after a time the virus becomes sufficiently potent to destroy a full-grown animal. Finally it regains its full activity, and will kill a sheep.

The form of induced septicæmia in the rabbit, which has been especially studied by the writer (see p. 354), furnishes a good example of an infectious disease resulting in one species of animal from the introduction into its body of a microorganism which is harmless for other species. This organism — a micrococcus — is commonly found in normal human saliva, where it is associated with various other species. Experiments thus far made indicate that there are various physiological varieties (breeds) of this micrococcus, varying in pathogenic power; for the saliva of different individuals differs in virulence. This may be accounted for by the fact that the conditions are not identical. The human mouth is a culture-apparatus in which the conditions are extremely favorable for the development of these minute plants; the secretions from the salivary glands afford a constant supply of pabulum, and the temperature is maintained at a fixed point. But the flow of saliva is more abundant in some persons than in others; and the presence of decayed teeth and of organic material, from neglect of the tooth-brush, may favor the development of putrefactive bacteria, which are fatal to the species of micrococcus which produces septicæmia in rabbits. Differences in habit as to the expectoration of the saliva or retaining it in the oral cavity, and as to breathing through the nose or through the mouth, will also constitute differences in the environment of the micrococcus which can scarcely fail to have an influence upon its physiological characters. When the flow of saliva is rapid, and it is not long retained in the mouth, it is evident that an organism which multiplies rapidly will have the advantage of one which multiplies slowly and may survive where the other would quickly disappear. There will also be a constant tendency to develop still further this capacity for rapid multiplication, which no doubt is an important, if not the essential, factor in giving to a micro-organism pathogenic power. The importance of this factor will be appreciated when we remember that one method by which nature limits the power for mischief of putrefactive bacteria injected into the tissues is by a conservative inflammatory process, which builds a wall about the invading parasites, and confines their depredations within the narrow limits of an abscess. In the disease produced by inoculation with saliva, or with a culture-fluid containing the micrococcus under consideration, owing perhaps to the rapid development of the micrococcus, no such limiting wall of inflammatory exudation is established; and we find the subcutaneous connective tissue diffusely infiltrated with serum which swarms with the parasite.

The failure to restrict the inroads of the parasite may not be due alone to its power of rapid multiplication. It is not improbable that some poison is produced, during its active growth, which lowers the vital resistance of the tissues and prevents the occurrence of conservative adhesive inflammation. And it may be that the true explanation of the immunity afforded by a mild attack of an infectious germ-disease is to be found in an acquired tolerance to the action of a chemical poison produced by the micro-organism, and consequent ability to bring the resources of nature to bear to restrict invasion by the parasite.

In the infectious disease known as hospital gangrene, circumstances relating to the origin, nature, and treatment of the malady make it seem extremely probable that some species of bacterium, ordinarily harmless, develops pathogenic properties as the result of an unusually favorable environment, and becomes the infecting agent, which, by invading the enfeebled tissues, causes the rapidly extending necrosis which is characteristic of this frightful malady. This disease is developed *de novo* in the surgical wards of hospitals, where numerous patients, with profusely discharging wounds, are brought together. Like its congeners, erysipelas and puerperal fever, it is prevented by cleanliness and antiseptic treatment. Its progress cannot, however, be arrested by ordinary antiseptic applications; for the pathogenic organism (hypothetical as yet) invades the tissues to a certain depth, and its destruction requires something more than a superficial germicide action, — *e. g.*, bromine, nitric acid, the hot iron.

Diphtheria, also, is a disease in which there seems to be good reason for believing that the different degrees of virulence are due to circumstances relating to the genealogy of the infecting organism, as well as to the resisting power of the infected individual; and that, as in anthrax and in fowl-cholera, physiological varieties of the pathogenic micrococcus, to which this disease is probably due, may be developed by special conditions relating to its environment, either in the fauces of an infected individual or external to the human body.

It is not alone by invading the blood or tissues that bacteria exhibit pathogenic power. Chemical products evolved during their vital activity, external to the body, or in abscesses and suppurating wounds, or in the alimentary canal, may doubtless be absorbed and exercise an injurious effect upon the animal economy. Indeed, we have experimental evidence that most potent poisons are produced during the putrefactive decomposition of organic matter. The poisons, resembling the vegetable alkaloids in their reactions, called ptomaines by Selmi, who first obtained them from a cadaver, are fatal to animals in extremely minute doses.

These ptomaines have also been obtained by Gautier from putrid blood and from the normal secretions of healthy persons, — saliva, urine, blood, etc.

The soluble poison, sepsin, which has been shown by the researches of Bergmann, Panum, Burdon Sanderson, and others, to exist in putrid blood is fatal to animals when administered in a sufficient dose, which, however, is very small. According to Koch five drops of blood, which has not putrefied too long, is sufficient to kill a mouse within a short time. After receiving an injection of this kind the symptoms of poisoning are developed *immediately*, and the animal dies in from four to eight hours.

"In such a case the greater part of the fluid injected is found in the subcutaneous cellular tissue of the back in much the same condition as before it was injected. It contains bacteria of the most diverse forms, irregularly mixed together, and as numerous as when examined before injection. No inflammation can be observed in the neighborhood of the place of injection. The internal organs are also unaltered. If blood taken from the right auricle be introduced into another mouse, no effect is produced. Bacteria cannot be found in any of the internal organs or in the blood of the heart.

"An infective disease has therefore not been produced as the result of the injection. On the other hand, there can be no doubt that the death of the animal was due to the soluble poison, sepsin.

. . . .

"This supposition is confirmed by the fact that when less fluid is introduced into the animal the symptoms of poisoning which follow are less marked, and are quite absent when one or at most two drops have been injected."[1]

On the other hand, the infectious disease which results in certain cases from a similar inoculation produces death only at the end of forty to sixty hours, and is attended with definite pathological lesions and the presence of a minute bacillus in the blood and tissues of the infected animal. A very small quantity (e. g., one-tenth of a drop) of the fluid of the subcutaneous œdema, or of blood from the heart of such an animal, is sufficient to infect another, and Koch has fully demonstrated the infectious nature of the disease by a series of seventeen successive inoculations. He says: "It is sufficient, in order to bring about the death of the animal in about fifty hours, to pass the point of a small scalpel, which has been in contact with the infected blood, over a small wound in the skin."

This distinction between septic toxæmia and infectious septicæmia, which has been established by the experimental researches of Koch, Pasteur, and many others, is opposed to the results reported by Rosenberger of Wurzburg, who claims to have demonstrated that the various forms of septic micro-organisms appear in the body of an animal which has been subjected to experimental inoculation, not because like organisms have been introduced as seed, but as a result of the introduction of a chemical poison which causes organisms previously present in the body of the animal to make their appearance in the blood, etc.

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[1] Traumatic Infectious Diseases, Sydenham Society's translation, London, 1880, p. 35.

That the injection of sepsin favors the development of bacteria introduced at the same time is very probable, and we cannot help believing that Rosenberger has unwittingly introduced living bacteria with his cooked septic blood and serum, notwithstanding the precautions which he claims to have taken. This view is supported by the experiments of Zuelzer and Sonnenschein, who, finding a resemblance between the physiological effects of sepsin and of atropia, injected two to five centigrammes of neutral sulphate of atropia at the same time with a culture-solution containing bacteria. Fatal septicæmia was found to result from these inoculations, while the bacteria injected alone did no harm.

The subcutaneous injection of other potent poisons has been found to be followed by local necrosis and rapidly developed putrefactive changes; but there is reason to believe that in these instances, also, the putrefactive germs are introduced simultaneously with the chemical poison, or find their way through the inoculation wound from the exterior, rather than to suppose that they are developed within the body of the animal. For the observations and experiments of numerous investigators are opposed to the belief that bacteria are habitually present in the blood and tissues of living animals. They are known to infest the alimentary canal, and it is probable that the smallest portion of hair or epithelium detached from the surface of the body of any one of the lower animals would fertilize a culture-solution; but blood drawn from the veins with proper precautions does not fertilize a sterilized culture-solution. Koch says (*l. c.*) "I have on many occasions examined normal blood and normal tissues by means which prevent the possibility of overlooking bacteria, or of confounding them with granular masses of equal size; and I have never, in a single instance, found organisms. *I have, therefore, come to the conclusion that bacteria do not occur in the blood, nor in the tissues of the healthy living body, either of man or of the lower animals.*"

As an example of the development of putrefaction, as a result of inoculation by a chemical virus, we may refer to the recent experiments of Weir Mitchell, and Reichert, "On the Venom of Serpents." These gentlemen find that venom contains three proteids. One of these, venom peptone, is not poisonous as a venom, but its injection into the breast of a pigeon gives rise to remarkable local effects. A lump forms, and within forty-eight hours a gangrenous cavity is produced, from which putrefactive odors are given off. That putrefaction here, as elsewhere, is produced by the bacteria of putrefaction, there can be no doubt; for no known proteid is capable of producing putrefactive changes in a sterilized organic fluid; and that the bacteria of putrefaction were introduced from without, is likewise altogether probable, inasmuch as we have no account of special precautions having been taken to exclude these ubiquitous organisms, and in view of what has just been said as to their absence from the blood and tissues of healthy animals.

Panum found that a putrid solution boiled for eleven hours still produces symptoms of putrid poisoning, and that when such a fluid is evaporated to dryness, and the residue extracted, first with alcohol and then with water, the alcoholic extract does not produce the symptoms, while the watery extract does. There can be little doubt that the watery extract injected contained living bacterial germs, not from the putrid fluid operated upon, but in the water used for making the extract (cold), in the syringe used for injecting it, or possibly carried from the surface of the body of the animal by the point of the needle used in making the injection. According to this explanation, germs introduced in the way indicated would multiply and produce putrefactive decomposition because the vitality of the tissues was reduced or destroyed by the chemical poison; whereas if introduced alone, even in vastly greater numbers, they could do no harm, owing to the vital resistance of the tissues. The writer has frequently injected culture-fluids containing the bacteria of putrefaction beneath the skin of a rabbit, without serious result. But the smallest drop of fluid containing the oval micrococcus, which produces *infectious* septicæmia in rabbits, produces a fatal result within forty-eight hours; and the virulence of blood, or of a culture-fluid containing this micrococcus or the anthrax bacillus (without spores), is destroyed by exposure for ten minutes to a temperature of 140° Fahr., whereas sepsin, the ptomaines, and serpent virus — *venom globuline* — all withstand a boiling temperature.

In the pages which follow, the writer proposes to pass in review the infectious diseases which, upon evidence more or less convincing, have been supposed to depend upon the invasion of the infected animal by a parasitic micro-organism. The limits of the present volume will, however, only admit of a brief *résumé* of the observations and experimental evidence bearing upon this supposition, for each disease in the list; and the reader who desires fuller information, is referred to the copious bibliography appended. For convenience of reference, the diseases are arranged alphabetically.

PLATE X.

Copied from photographs by Koch in Cohn's "Beiträge zur Biologie der Pflanzen," Bd. II., Heft 3.

FIG. 1. — *Bacilli* of Miltzbrand from the substance of the spleen. × 700.

FIG. 2. — *Bacilli* of Miltzbrand in blood of basilar artery collected two days after death. × 700.

FIG. 3. — *Spirillum Obermeieri.* × 700.

FIG. 4. — *Bacilli* of Miltzbrand cultivated in aqueous humor, showing development of spores. × 700.[1]

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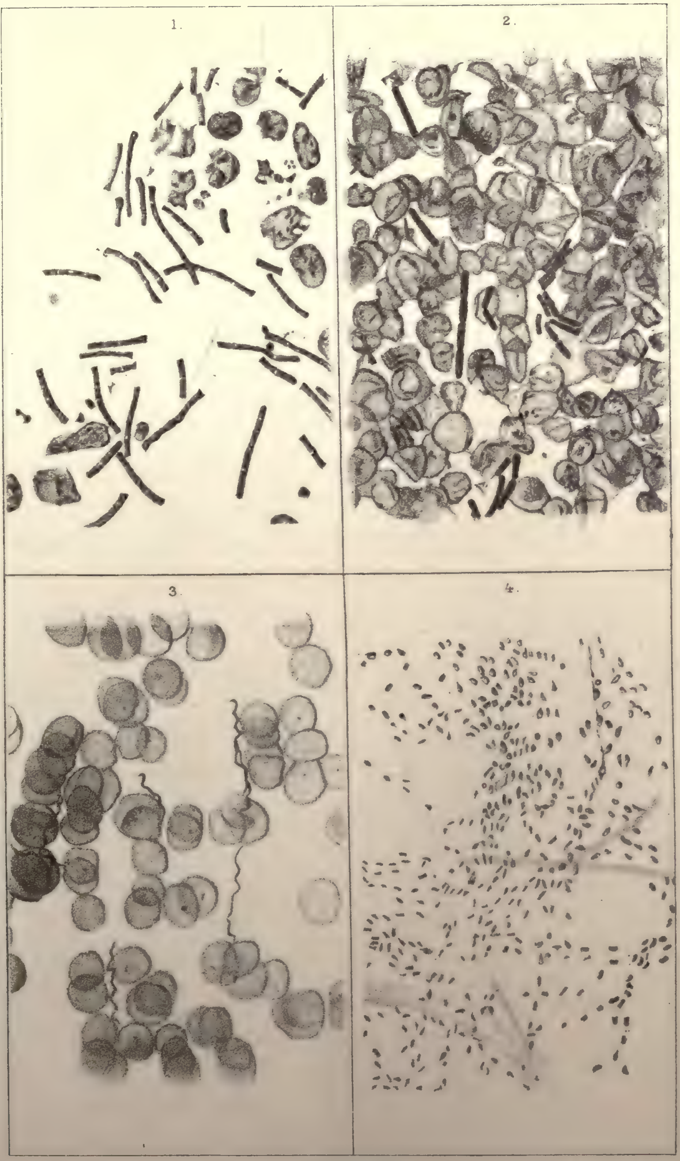
[1] The lithographer has not succeeded in making a satisfactory copy of Koch's photo-micrograph, in this figure.

Plate II.



A Meisel, lith.

INFECTIOUS DISEASES WHICH HAVE BEEN ASCRIBED TO THE PRESENCE OF BACTERIA.

ANTHRAX; *Charbon*, Fr., *Miltzbrand*, Ger. — This is an infectious disease of animals which may be transmitted to man by inoculation. This occurs, occasionally, from the bite of an insect (fly) which has been feeding upon the carcass of an infected animal; and also from accidental inoculation while handling hides, wool, etc., taken from the victims of anthrax.

The herbivora are most susceptible to anthrax; and in certain parts of Europe the annual losses from this disease, among the herds and flocks of the farmers, are very considerable.

The susceptibility of the carnivora to this and other forms of septicæmia is very much less than that of the herbivora. This difference is probably due to natural selection; for the bodies of herbivorous animals, dead from anthrax, have doubtless been devoured by the carnivora from the earliest times (anthrax was known to the Greek and Roman physicians); and, although inoculation is not liable to occur through the uninjured mucous membrane of the mouth, or of the intestine, it could scarcely fail to occur as a result of wounds inflicted by the teeth and claws of the contestants for the infected prey. An individual difference in susceptibility to the poison, and the survival of the fittest, would in time be very sure to produce a race immunity. This view is not, however, sustained by the experiments of Prof. Feser upon rats. In these experiments it was found that rats fed on flesh do not contract anthrax, but that the same rats when restricted to a vegetable diet fall victims to the disease after inoculation with anthrax fluids.

The immunity of fowls has been proved by Pasteur to be a question of temperature. According to Chauveau, multiplication of the bacillus in culture-fluids ceases at 43°. This is but little above the normal temperature of the fowl. If, however, the temperature is reduced two or three degrees by immersing the lower part of its body in cold water, the fowl becomes susceptible and dies as the result of inoculation with a fluid containing the bacillus.

The anthrax bacillus is said to have been observed by Pollender in the blood of cattle as early as 1849, and by Davaine in 1850. But the etiological importance of the parasite was first recognized by the last named observer, and was affirmed in a series of communications to the French Academy, made in 1863 and 1864. The experiments of Davaine established the fact of the presence of rod-shaped bacteria in the blood of animals attacked with *charbon*, and that a healthy animal into which a small quantity of this blood is injected quickly succumbs to the disease, its blood also being invaded by the parasite.

The view that the infectious properties of anthrax blood depend upon the presence of this parasite was strongly contested, and since Davaine's first experimental inoculations, a host of investigators have entered the field. The question is admitted by all to be of the greatest importance, and has been most thoroughly investigated by the experimental method, every point made by those in favor of the parasite-germ theory having been stoutly contested by conservative opponents. The literature of the subject, although so recent, is very voluminous; and the fact that the anthrax bacillus is the essential infectious element in anthrax blood, and that the disease anthrax is due to the multiplication of this parasite in the body of an infected animal, has been established in the face of the most exacting scientific criticism.

Klebs first showed that anthrax blood loses its infectious properties after filtration, while the filtrate is virulent; but as other solid elements (fibrine and globules) were retained as well as the bacilli, this was not accepted as proof that the latter were the essential infectious particles.

This proof has been furnished by inoculation experiments with pure-cultures of the anthrax bacillus, which have now been made by numerous experimenters in various parts of the world. By successive cultures, in which a small amount of material is used to inoculate a considerable quantity of the culture-fluid, we soon exclude all non-living particles, and soluble substances as well, contained in the material introduced as seed into culture No. 1 (see remarks on p. 238).

In such a series, which has been carried as far as the one-hundredth successive culture (Pasteur), the virulence of the last culture-fluid is as great as that of the first; and, as the culture-fluid itself is innocuous, this virulence can be ascribed only to the living bacilli contained in it, which are the direct descendants of those present in the minute drop of anthrax blood used to inoculate culture No. 1.

Experiments of this kind are conclusive as to the essential etiological *rôle* of the anthrax bacillus, but they do not, of course, explain its *modus operandi*. Pasteur has shown that the bacillus is *aérobie*, — i.e., that its development depends upon the presence of oxygen, — and there can be no doubt that, during its rapid multiplication in the blood of a living animal, it deprives this fluid of its oxygen, and also of other constituents required for its own nutrition. The deprivation of oxygen is shown by the symptoms, — dyspnœa, cyanosis, depressed temperature, and finally death, with all the symptoms of asphyxia. It also acts mechanically, by blocking up the capillaries, and producing emboli and hemorrhagic extravasation in various parts of the body. In addition to this, we have evidence that, as in other forms of septicæmia, a potent chemical poison is produced as a result of vital processes connected with the nutrition of the bacillus. Paul Bert has been able to isolate a poison, diffusible in liquid, which kills in twelve hours. This he accomplished by destroying the bacillus in a fluid containing it by means of compressed oxygen. Toussaint, also, by injecting filtered anthrax blood, obtained evidence of the presence in it of a poison which, in his experiments, produced only a local inflammation, without any noticeable constitutional symptoms.

The discovery, which we owe to Koch, that, under favorable conditions, the anthrax bacillus, either in culture-fluids or in the body of a dead animal, develops refrangant, endogenous spores, which have great resisting power against heat and chemical reagents, and may be preserved for years without loss of vitality, has enabled us to account, in a most satisfactory manner, for certain facts which previously seemed to be irreconcilable with a belief in the parasitic-germ theory. Thus Bert treated anthrax blood, which he had received from Alfort, with three times its volume of absolute alcohol, then washed the coagulum in alcohol, and dried it *in vacuo*. This material, mixed with water and again precipitated by alcohol, proved to be virulent when injected into guinea-pigs. Even after remaining for five months immersed in alcohol, this virus had not lost its potency.

These facts were explained by Pasteur, and, in a subsequent communication, Bert himself explained the mystery. Further experiments had convinced him that virulent fluids containing anthrax rods did not resist either alcohol or compressed oxygen, and that it was only when reproductive spores were present that the flakes of material precipitated by alcohol gave evidence of virulence. Upon microscopical examination these shining spores were detected in the flakes in question, and their continued vitality after the treatment indicated was proved by their germination in a culture-fluid.

The anthrax rods are killed by ten minutes' exposure to a temperature of 54° C. (129°.2 Fahr.), by desiccation, and by putrefaction of the fluid containing them, in the absence of oxygen; but the resting-spores resist prolonged boiling (Pasteur), and are not injuriously affected by desiccation or by putrefaction. Spores are not formed in the rods as they are found in the body of a living animal; but after death, under favorable circumstances, these rods grow into filaments in the interior of which shining oval bodies are developed, which are the spores in question. Thus the carcass of a dead animal may become a storehouse of anthrax seed, which may for many years after its death infect pastures in which the animal was buried. But no development of spores occurs in the absence of oxygen; and under these circumstances the rods quickly disintegrate and disappear. This is shown by enclosing in a tightly corked bottle blood from an animal recently dead. Putrefactive decomposition soon takes place, but the blood loses its virulence, and neither rods nor spores can be discovered in it after a few days.

According to Ewart, when cultivated upon a warm stage in albuminous fluids, the anthrax rods become motile within a few hours, and exhibit alternations of motion and quiescence. This does not correspond with the observations of Koch, and is probably a mistake. Magnin, on page 88 of the present volume, in giving the specific characters of *B. anthracis*, states that it is always motionless. If the temperature is maintained at about 33° C. (91.4° Fahr.) the rods soon grow into long homogeneous filaments, which in the course of four or five hours may reach a length many times greater (50–100 times) than the original bacilli. These are often twisted and interlaced in the culture-fluid. A little later the filaments, which were at first hyaline, are seen to consist of a distinct sheath and a central cylinder of protoplasm, which soon undergoes segmentation, each segment being about the length of the original rods. These segments, and the rods themselves as found in blood, break up into smaller masses under the influence of staining reagents. This is seen in my photomicrograph, Fig. 1, Plate XI. The spores are formed by a consolidation of the protoplasm of one of these segments into an oval mass, which is subsequently set free by rupture of the cellular envelope, or by its granular disintegration. The oval shining spores after their escape present the appearance of being enclosed in a gelatinous en-

PLATE XI.

FIG. 1.—*Bacillus anthracis* from liver of mouse. The spherical objects are fat globules from the crushed liver tissue. The longer filament shows transverse septa, referred to by Koch. × 1000; Zeiss's $\frac{1}{18}$ in. objective. Methyl-violet staining.

FIG. 2.—The same bacillus (*B. anthracis*) from liver of mouse. × 500. Aniline brown staining.

FIG. 3.—Epithelioid cell containing several of Koch's *Bacillus tuberculosis*. From sputum of phthisical patient, stained by Ehrlich's method. The bacilli and the outlines of the cell containing them can scarcely be perceived. (See remarks on p. 196.) × 1000 diameters; Zeiss's $\frac{1}{18}$ in. objective. Fuchsin staining.

FIG. 4.—Spore-bearing filament of *B. anthracis*, from culture in chicken *bouillon*. Scattered spores and fragments of filaments which had broken up without producing spores, are also seen. × 500 diameters. Methyl-violet staining.

FIG. 5.—Bacilli from surface of gutter-water; Baltimore, 1881. × 1000 diameters. Methyl-violet staining.

FIG. 6.—Tubercle bacillus from sputum of phthisical patient. A close inspection will show that it contains spores. (See remarks on p. 394.) × 1000 diameters by Zeiss's $\frac{1}{18}$ in. objective. Stained with fuchsin by Ehrlich's method.

FIG. 7.—Bacilli from pleural cavity of rabbit, which suffered an attack of acute pleuritis, with fibrinous and serous exudation, as a result of an experimental inoculation with contaminated water (Water No. 5, Professor Mallet's experiments). The source of contamination is unknown to the writer, and the figure is simply introduced to show another species of bacillus, possibly pathogenic, and to call attention to its resemblance to the bacillus shown in Fig. 8. × 1000; Zeiss's $\frac{1}{18}$ in. Methyl-violet staining.

FIG. 8.—Bacillus from normal human saliva; culture-experiment in acid malt-extract solution. × 1000; Zeiss's $\frac{1}{18}$ in. Methyl-violet staining.

Plate XI.

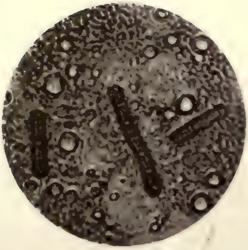


Fig. 1.

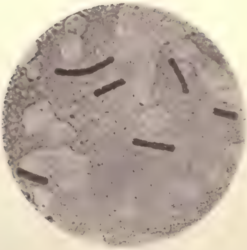


Fig. 2.

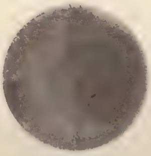


Fig. 3.

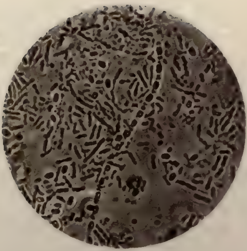


Fig. 4.

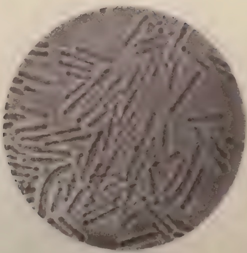


Fig. 5.

Fig. 6.

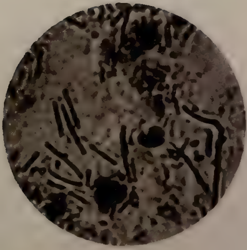


Fig. 7.

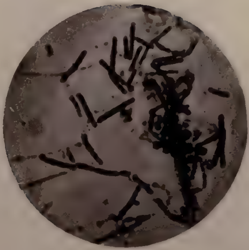


Fig. 8.

velope, which, according to Koch, is developed into a new rod when germination takes place. Other observers (Ewart, Cohn) assert that the central protoplasm is developed into a new rod, and that the envelope is used up during its growth.

At 35° C. (95° Fahr.) spores make their appearance at the end of twenty-four hours. At a lower temperature (28° C.) the growth of the rods into filaments takes place more slowly, and the formation of spores is not completed in less than thirty-six to forty-eight hours. At 42° to 43° C. the rods grow and multiply by fission, but spores are no longer formed. No development occurs at temperatures below 12° C. (53.6° Fahr.). In Fig. 3, Plate XI., a spore-bearing filament is seen in the centre of the field, while scattered about are liberated spores and detached segments of the filaments. The amplification is 500 diameters, and the specimen is from a culture made in chicken *bouillon.*

A statement relating to the source of the material which furnished specimens for my photomicrographs, Figs. 1, 2, and 3, Plate XI., may not be uninteresting as illustrating the facts already given.

While pursuing certain experimental inquiries in the biological laboratory of Johns Hopkins University during the summer of 1881, Professor Martin placed in my hands a small tube just received by him from Dr. Burdon-Sanderson, of

London. In a letter accompanying this, Burdon-Sanderson says: "I send you the material I started from in the last experiments I made upon the subject (anthrax). It was then five years old, and consequently is now seven or eight. I have no doubt that you will find that if worked up with salt-solution and injected into a mouse, you will have the spleen — after from twenty-four to thirty-six hours — enlarged and infiltrated with Bacillus." This scientific prediction was fulfilled to the letter. The little tube only contained a fraction of a grain of dried blood. This was rubbed up with a little salt-solution in accordance with the directions given, and a few minims of the solution injected beneath the skin of a recently-captured mouse. The animal died in a little less than thirty-six hours, and its liver and spleen contained an abundance of bacilli, which are shown in my photo-micrographs (Figs. 1 and 2, Plate XI.).

A portion of the spleen of this animal was placed in a culture-cell with a little chicken *bouillon*, and kept for twenty-four hours in the culture-oven, at a temperature of 100° Fahr. The following day the culture-fluid was found to contain a luxuriant growth of filaments, many of which contained shining oval spores (see Fig. 3, Plate XI.). A fragment of the spleen of the mouse was used to inoculate a small quantity of blood from a healthy rabbit, drawn directly into a sterilized tube. The anthrax bacillus multiplied abundantly in this blood, growing into long filaments and forming spores, as in the culture in chicken *bouillon*. On the 13th, two minims of this blood-culture were injected into a small rabbit, and a still smaller quantity into another mouse. The mouse died on the following day, and the rabbit on the 16th. Upon post-mortem examination an abundance of bacilli were found in the blood, liver, and spleen of both of these animals. My only object being to obtain a stock of anthrax virus, and material from which to make some photo-micrographs of *Bacillus anthracis*, the experiments were not pursued any further.

If we were without satisfactory experimental evidence that the *Bacillus anthracis* is the cause of the disease anthrax, we could scarcely suppose any longer that its presence in this disease is without import, a mere epi-phenomenon, in the face of such evidence as that given by Koch in the following extract from his work on "Traumatic Infective Diseases" (Sydenham Society's translation): —

> "Although I had often previously examined the blood of animals suffering from anthrax, and had thus formed a high estimate as to the number of bacilli present in the body of an anthracic animal, yet I was quite surprised when I saw for the first time sections and portions of organs stained in this way [in methyl-violet, with carbonate of potash, see p. 187], as *e. g.*, the intestinal mucous membrane and the iris of a rabbit. When magnified fifty diameters, such a preparation

presents, at the first glance, an appearance as if a blue coloring matter had been injected into the vessels. Each intestinal villus is permeated by an exceedingly delicate blue net-work; in the mucous membrane of the stomach all the capillary net-work surrounding the gastric glands is stained blue; in the ciliary processes each projection is injected, and a spiral vessel stained of a dark blue color leads from thence to the iris, and breaks up into a fine blue net-work with loops directed towards the edge of the iris. The liver and lungs, and the glandular structures, such as the pancreas and salivary glands, are completely permeated by the same blue capillary net-work. Indeed there is no organ which is not more or less injected with the blue mass. It is, however, very striking that this injection is only present in the capillary vessels. All the larger vessels, even the arteries and veins of an intestinal villus, are either not stained at all or have but a light blue streak in their interior, and that only here and there. When magnified 250 times one can see that the blue capillary net-work is composed of numerous delicate rods, and when a power of 700 diameters is used, it is found that the apparent injection is nothing more or less than the *Bacillus anthracis*, stained dark blue, and present in incredible numbers in the whole capillary system. In the other vessels, especially in the larger ones, often only a single bacillus may be met with at long intervals, or they may be quite absent. Here, therefore, we have a striking proof of how little value are conclusions drawn in traumatic infective diseases from the examination of a drop of blood taken from a blood-vessel by chance; for one might well take a drop of blood from the heart and find no micro-organisms in it, or one might readily overlook the few which might be present, and that although the capillary system abounds in these."

The results obtained by Pasteur in his experiments relating to protective inoculations against anthrax, have been of the highest importance, and many persons have been led to share in the sanguine expectation of the distinguished French chemist, that not only in this disease, but in the infectious diseases generally, protective inoculations may eventually be successfully practised. The various methods of effecting "attenuation of virus" have been described in PART THIRD of the present volume.

Pasteur recommends, in anthrax, a double inoculation, first with a greatly mitigated virus, *premier vaccin*, and subsequently with a more potent virus, *deuxième vaccin.* Whether in practice it will be found wise to resort to protective inoculations rather than to attempt to stamp out the disease by the destruction of infected animals and other vigorous preventive measures, is open to question. As Klein has pointed out, the method of Pasteur involves a multiplication of the poison, which may add to the danger of extensive losses occurring among herds and flocks which have not been protected. Moreover, there is a certain mortality from the application of the method, and we have not yet learned how durable the protection may be. While, therefore, we accord full honor to Pasteur for his valuable contributions to science in connection with this interesting subject, we must admit that, as a practical measure of protection, the method is still under trial. Koch is not at all sanguine as to the possibility of extending the application of the method to other infectious diseases, and points out that even in anthrax no general law of immunity has been established; as several observers (Lœffler, Gotti, Guilebeau, and Klein) have shown that no such immunity is obtained in the case of guinea-pigs, rats, mice, and rabbits, and that thus far only sheep and cattle have been proved to acquire immunity from inoculations with attenuated virus.

An interesting question, which has not yet been definitely decided by experiment, relates to the possible protection of an animal which has suffered an attack of one form of septicæmia — e. g., anthrax — from the other allied forms. Certainly the infectious septicæmia of rabbits, due to a micrococcus, which the writer has especially studied, bears a strong resemblance, in many particulars, to anthrax, and the same may be said of the form of septicæmia in mice, due to a minute bacillus, which has been described by Koch. The question is whether an animal which has recovered from a modified form of one of these diseases will not be protected from the others. If so, it is extremely probable that protection results from tolerance to the chemical poison evolved during the growth of the micro-organism, and consequent ability on the part of the tissues to withstand the attacks of the parasite, rather than to the using up of some material in the body of the animal which is essential for the development of the *microbe*. In this case the chemists are likely to find that the poison present in the blood of animals suffering from these different forms of septicæmia is the same, although the *microbes* differ. According to Pasteur "there are as many different forms of septicæmia as there are different vibrios."[1] And in a letter to his *confrère*, Dumas, he says: "Numerous experiments have shown me that cultivation of the *bactéride* (*Bacillus anthracis*) in a medium exhausted by the microbe of fowl-cholera, although real, is retarded, not abundant, and difficult. Contrary to the provisions which I have just recalled, it may be, then, that fowls vaccinated for cholera are refractory to charbon, which is due to a parasite of quite a different nature. Such is precisely the unexpected result which I have obtained in some experiments not yet sufficiently numerous to prove the fact."

In a later communication,[2] Pasteur says: "It may be considered as established: *First.* That chickens are refractory to charbon. *Second.* That chickens, when refrigerated, easily contract charbon. *Third.* That chickens in which charbon is established by a lowering of temperature, may be completely cured by warming them."

According to Arloing, Cornevin, and Thomas, immunity from anthrax does not protect from the disease which they have studied and call symptomatic anthrax; nor does immunity from the latter disease afford protection against the former.

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[1] Charbon and septicæmia. *Comptes rendus* LXXXV.

[2] *Comptes rendus* LXXXVII., p. 47.

An important question, which has received the attention of several investigators, relates to the possibility of the passage of the bacillus from the circulation of a pregnant female, through the placenta, to the fœtus *in utero*. It is well known that the placenta does not permit of the passage of blood-corpuscles, and experimenters very justly reasoned that if the blood of the fœtus of an animal which has succumbed to an attack of anthrax is free from bacilli while the mother's blood contains them, inoculation experiments with this blood should furnish strong evidence for or against the germ theory.

The observations of Brauell, of Davaine, and of Bollinger, were all in accord as to the absence of the bacilli from the blood, and its non-virulent character. But, more recently, Strauss and Chamberland have shown that there are some exceptions to this rule, and that occasionally the fœtal blood contains a few bacilli. This was proved by culture experiments, and when the bacilli were present the blood was found to be virulent, when injected in sufficient quantity.

SYMPTOMATIC ANTHRAX; *Charbon symptomatique.*—This disease, according to Arloing, Cornevin, and Thomas, is characterized by the presence of a microbe which has distinct morphological characters, and which differs essentially from the anthrax bacillus. It is shorter and broader than *B. anthracis*, is rounded at the extremities, is extremely mobile, and is nearly always provided at one extremity with a refractive spore. Sometimes the rod is very long and has a spore at each extremity. It may happen that the microbe is only distinguished by this spore, as the rod has nearly the same refractive index as the fluid in which it is found. The writer would remark, *en passant*, that he has observed bacilli which answer very well to this description, in putrid blood, and especially in a specimen of blood sent to him from Havana, which had become putrid *en route*. This was obtained from a yellow-fever patient, *post mortem*. Photo-micrographs were made of this organism, and heliotype reproductions of these are seen in Figs. 1 and 2, Plate VIII., of the present volume. These bacilli are endowed with active motion, have rounded extremities, and very commonly contain a highly refractive spore at one end, as seen in Fig. 2.

According to the authors named, symptomatic anthrax occurs especially in young cattle, of six months to four years, and in lambs. It is characterized by loss of appetite, debility, and lameness due to the development of a tumor. Wherever situated, this tumor is irregular in form, and extends in every direction with astonishing rapidity. In eight to ten hours it attains an enormous development. At first homogeneous and extremely painful, the tumor becomes, little by little, insensible in the centre and crepitates on pressure. All of the tissues forming this tumor are black and friable. When incised, bright red blood escapes, in the earlier stage of development, later a liquid resembling venous blood, and at last a frothy serum. The tumor may be deeply buried in the muscles, and may then escape observation. Death usually occurs within 36 to 48 hours after the appearance of the first symptoms. The disease is always fatal. After death the body rapidly becomes inflated by an accumulation of gas in the abdomen, in the veins, and in the cellular tissue. One or more bloody tumors are found among the muscles, which, when incised, present a characteristic black color, are very friable, and infiltrated with gas. The digestive organs are usually entirely healthy, and the liver and spleen are normal in appearance although they contain the microbe in abundance.

Symptomatic anthrax is not readily communicated by small amounts of virus. In a few cases only have successful inoculations been made with the pulp of diseased glands in small quantity. But larger amounts of the infectious material produce the characteristic tumors. In susceptible animals a few drops of blood, or muscle pulp, forced into the cellular tissue produces fatal results. Intra-venous injections of as much as 2.6 c.c. of the pulp from a tumor are tolerated by the calf, sheep, and goat. A mild sickness results from such an injection, and in rare cases death occurs. The guinea-pig is susceptible, but the rabbit is not. The microbe is said to present different characters in the blood, in the tumors among the muscles, and in the effused serum in the connective tissue.

Immunity is said to result from intra-venous injection of material containing the microbe. Subsequent sub-cutaneous injection of pulp from a tumor produces no results in these animals. The value of this method has been tested by experiments upon 244 animals, made under the authority of the French Government. The results of intra-venous injection differ with the amount of virus employed. When the quantity is very small, general disturbances are produced which disappear in two or three days, leaving the subject immune. When the dose is considerable, fatal symptomatic anthrax is produced. The experimenters suppose that in non-fatal intra-venous injections the bacterium multiplies in the blood, but is prevented by the *endothelium* of the vessels from entering the connective tissue.

Filtration experiments show that the poison is particulate, and the authors quoted claim to have proved that the bacterium described by them is the veritable cause of the disease. The experiments recently made by the same authors to determine the comparative value of disinfectants for the destruction of this virus, seem to support their deductions as to the essential etiological *rôle* of the microbe.

The preservation of virulence after exposure to sulphurous acid, to alcohol saturated with camphor, etc., is accounted for by the presence of spores. This corresponds with Koch's results as to the resisting power of the spores of *Bacillus anthracis*, which, it will be remembered, are not found in the bacilli as they occur in the blood and tissues of a living animal. The superior resisting power, as regards retention of virulence, of the fluids of symptomatic anthrax to an elevated temperature is also, no doubt, due to the presence of spores. In a recent series of experiments Arloing, Cornevin, and Thomas have determined the thermal death-point of these spores. Fresh virus lost all pathogenic power when heated for two hours at 80° C., or by subjection to a boiling temperature for twenty minutes. An attenuated virus of different degrees of power could be produced by subjecting the material to a temperature lower than that which destroyed it entirely. Thymol and oil of eucalyptus were capable of attenuating the virus in forty-eight hours, without destroying the vitality of the microbes.

In symptomatic anthrax, contrary to the usual rule in anthrax, the fœtal blood is virulent, and contains the bacteria to which this virulence is ascribed.

CEREBRO-SPINAL MENINGITIS.—Leyden reports the finding of "oval micrococci, in great numbers, occurring both singly and in chains," in recent lymph obtained by a hypodermic syringe from beneath the pia mater of the spinal cord in a sporadic case of this disease.

Cholera. — Epidemiologists find it necessary to assume the existence of a living germ in order to explain in a satisfactory manner the origin and epidemic extension of this disease. Evidently the *materies morbi* is capable of self-multiplication external to the human body; and this multiplication is conditioned by circumstances of the same kind as those which influence the development of the lowest organisms, — heat, moisture, and the presence of organic material to serve as nutritive pabulum for the hypothetical germ.

Various attempts have been made to find the cholera germ in infected atmospheres and in the discharges of cholera patients, but thus far no satisfactory results have been attained. Modern methods of research have not, however, been fairly brought to bear in the investigation of this disease. The difficulties connected with such an investigation are very great, for the reason that a multitude of harmless micro-organisms are constantly present in the discharges from the bowels of healthy persons, and of cholera patients as well; and because no one of the lower animals has yet been proved to be susceptible to the disease. Consequently, there is no way of demonstrating the pathogenic potency of a suspected organism, which might be isolated by culture experiments. Magendie, Meyer, Lindsay, and others, have introduced material from the stools of cholera patients into the stomach, the veins, and the subcutaneous connective tissue, of various animals, with negative results. According to Lebert, putrefaction of cholera stools diminishes their capacity for infection, and the bacteria of decomposition destroy the germs of cholera. This is an inference drawn from the fact that those who, during a cholera epidemic, occupy themselves in making post-mortem examinations are not especially liable to be attacked with cholera.

ERYSIPELAS. — The infectious nature of erysipelas has been abundantly demonstrated, both by clinical and experimental evidence. The transmission of vaccinal erysipelas from one child to several others, and the communication of the disease by instruments previously used in dressing erysipelatous wounds, has been noted by physicians. Orth has also shown, by a series of twenty-three experiments, that the disease may be communicated by inoculation from man to the lower animals.

Numerous observers — Hüter, Nepveu, Wilde, Orth, Wahlberg, and others — have noted the presence of micrococci in the inflamed tissues, and especially in the œdema of erysipelas.

Fehleisen has recently given strong experimental evidence in favor of the pathogenic *rôle* of these micrococci. Not only has he demonstrated their presence in every case of erysipelas examined by him (13 cases), but he has succeeded in cultivating them, and has successfully inoculated men and animals with the cultivated micro-organisms. The micrococci were very numerous in bits of skin excised from the diseased surface in cases of erysipelas, and were commonly arranged in chains. They were never found in the blood-vessels, and were most numerous in recently affected parts; here they invaded the superficial layer of the corium and the sub-cutaneous adipose tissue, filling the lymphatics and the lymph-spaces. Fehleisen succeeded in cultivating these micrococci by placing bits of excised skin upon the surface of a jellified solution of gelatine. Here they produced by their abundant multiplication a whitish film, which was easily detached, and was composed entirely of the organisms. Nine rabbits were inoculated, and in eight a characteristic erysipelatous rash was developed after 36 to 48 hours. This was attended with febrile disturbance at the outset. In a few days the disease ran its course and the animals, without exception, recovered. The inoculations were upon the ear, both with micrococci taken directly from a patient, and with cultivated organisms. The disease extended from the point of inoculation to the root of the ear, and thence to the head and neck.

In one case the ear was amputated during the height of the disease, and the presence of micrococci demonstrated in the lymphatics of the affected part.

Fehleisen also inoculated the pure, and cultivated, organisms upon man, with a successful result in six out of seven cases. The period of incubation was from fifteen to sixty hours, after which rigors, followed by fever, occurred; and the typical erysipelatous rash developed itself, and ran the usual course.

CHOLERA OF FOWLS, *choléra des poules.* — Pasteur has furnished satisfactory experimental evidence that this infectious disease of the domestic fowl is due to a micrococcus, which he has cultivated for successive generations in *bouillon* made from the flesh of a chicken; but which does not multiply in yeast-water, a culture-medium well suited for the development of many species of bacteria.

Inoculation of healthy fowls with a pure culture of this micrococcus gives rise to the disease; but not invariably, as a marked difference in susceptibility exists in different individuals. Out of eighty fowls inoculated by Salmon, six recovered, twenty-five were not visibly affected, and forty-nine died.

One attack protects from subsequent attacks, and protective inoculations may be practised with "attenuated virus" prepared by Pasteur's method, — long exposure to oxygen.

The most potent virus is that obtained from a fowl which dies from a chronic form of the disease. In these cases, "the fowl, after having been very sick, grows thinner and thinner, and resists death for several weeks or months. When it perishes, which occurs shortly after the parasite, located previously in certain organs, has passed into the blood and increases there, we observe that whatever may have been the original virulence of the virus at the time of inoculation, that taken from the blood of the dead fowl has a considerable virulence and kills ordinarily ten times out of ten, twenty out of twenty."[1] When an interval of three to eight months is allowed to elapse between successive cultures, the virulence is modified more or less according to the length of time. But each degree of attenuation may be preserved through a series of cultivations made at short intervals (Pasteur). It is necessary to cultivate the micrococcus in contact with the air as it is *aérobie*. In Salmon's experiments it was observed to form a mycoderma upon the surface of the culture-fluid. The last-named observer states that the micrococci are not abundant in the blood of a fowl, drawn from a vein during life, but that they are more abundant in blood taken from the body after death. Putrefaction destroys the potency of virulent fluids. The thermal death-point of the micrococcus has been fixed by Salmon as somewhere between 124° and 140° Fahr. Of three fowls inoculated with virulent blood heated to 124° for fifteen minutes, two died; while two fowls inoculated with blood that had been heated to 140° for the same time, remained in good health; one was subsequently proved to be susceptible by inoculation with active virus, but the other resisted such inoculation.

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[1] *Comptes rendus* XCI., p. 375.

This corresponds very closely with the thermal death-point of the micrococcus of septicæmia in the rabbit, and the micrococcus of pus, as determined by the writer, and is strong evidence in support of the view that the virulence of the fluid containing it depends upon the vitality of the micro-organism. The resistance to various chemical reagents, as determined by Salmon, also corresponds very closely with results obtained by the writer in similar experiments with the micrococcus of induced septicæmia in the rabbit. These two species of *Micrococcus*, however, have distinct physiological properties, as the writer has proved the innocuousness of the oval micrococcus of septicæmia in the rabbit when injected into the muscles of fowls. Pasteur finds no difference, morphologically, between the organism which produces the "new disease" described by him (see p. 365), and that which produces *choléra des poules*. He also found that this micrococcus of induced septicæmia in the rabbit does not produce the slightest ill-effect when injected into fowls. Toussaint has claimed that fowl-cholera and acute septicæmia in animals, produced by the injection of the blood of animals dead with cholera, or of animal matters more or less putrid, are identical diseases and due to the same parasite. He says: "Since the experiments of Coze and Feltz in 1866, of Davaine, Vulpian, Bouley, etc., and the labors of the German *savants,* it is demonstrated that certain animal matters undergoing putrefaction, inoculated under the skin of the rabbit and of some other animals, produces, after several inoculations, a malady, rapidly fatal, inoculable with extremely minute quantities, and which reproduces itself indefinitely. The presence of a parasite in septicæmia which presents this character, has been sustained, then denied. . . . I can truly say, after several series of experiments comprising more than two hundred and fifty cases, that in the malady of rapid form which kills the rabbit in ten to twenty hours, and which is inoculated so easily into birds, there exists a microbe of well-determined form and properties, of which the action is always identical, which is that which Pasteur has so perfectly studied, and of which I have already demonstrated the presence in chicken cholera."[1]

DIPHTHERIA.—The presence of micro-organisms, and especially of micrococci in diphtheritic exudations, has been observed by numerous investigators, and was *a priori* to have been expected, inasmuch as the healthy human mouth is constantly infested with micrococci and other forms of bacteria. Oertel says: "They were discovered as far back as 1868, by Buhl, Hueter, and myself, in false membranes, the blood, and the tissues; in like manner they were demonstrated by Von Rechlinghausen, Wassiloff, Waldeyer, Klebs, Eberth, Heiberg, and others, in the most different organs and tissues."[1] The author quoted is very positive as to the etiological *rôle* of these micrococci, and agrees with Eberth in the statement, "*Without micrococci there can be no diphtheria.*" The *Micrococcus diphtheriæ*, Oertel, is described as follows, in a later work:[2] "It has an oval form, with a length of 1–1.5 μ, and a breadth of 0.3 μ; larger individuals, found nearer the surface, being 4.2 μ long, and 1.1 μ broad. Where the individuals are more scattered, they occur mostly in pairs, rarely a number connected into a torula-like chain. When present in masses, the cells lie so close together that it is difficult to determine whether they are connected or not. They are then imbedded in a gelatinous envelope, and thus combined in masses into a colony."[3]

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[1] *Comptes rendus* XCI., p. 302.

The inoculability of the disease has been proved by experiments upon animals; and filtration experiments (Eberth) show that the infectious element in diphtheritic exudation is particulate. Klebs, who has the credit of first resorting to the method of "fractional cultivations," claims to have produced diphtheria in animals by inoculating them with pure-cultures of the micrococcus, and to have subsequently recognized the parasite in their blood and tissues.

According to Ewart and Simpson, the pathogenic organism of diphtheria is a minute spore

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[1] Cyclopædia of the Practice of Medicine, Ziemssen, Vol. I. p. 590.

[2] Zur Aetiologie der Infectionskrankheiten, 1881.

[3] Quoted from Journal Roy. Mic. Soc. Ser. II., Vol. II. p. 88.

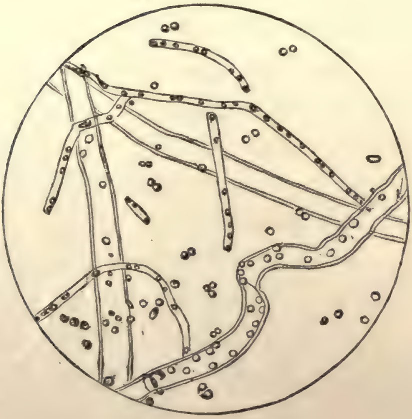

Fig. 10.

Copied from a photo-micrograph; amplification 1000 diameters.

which develops into long slender bacilli upon the surface of the tonsils, etc., when for any reason they are denuded of their superficial epithelium. The writer, while pursuing certain experimental investigations in Baltimore (1881) received, from Dr. H. C. Wood, material from the fauces of patients suffering from diphtheria, and also from scarlet fever patients, and made photo-micrographs of the micro-organisms found in the various specimens.

In the specimens marked "scarlet fever material," slender filaments, containing endogenous spores, were found, which correspond with those described by the writers named as peculiar to diphtheria. (See Fig. 10.)

Letzerich differs from other German observers in regarding a true fungus, of the hyphomycetous family, as the specific contagion of diphtheria.[1]

In the same "scarlet fever material," above referred to, the mycelium of a hyphomycetous fungus was found (Fig. 10), and also groups of spherical bodies which seemed to be the spores of a fungus of this nature (Fig. 11).

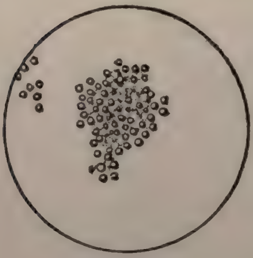

Fig. 11.

Copied from a photo-micrograph; amplification 1000 diameters.

Numerous micrococci were found in the specimens marked "diphtheritic material, Ludington," which did not differ morphologically from those which the writer had previously cultivated and photographed from normal human saliva. (See Fig. 1, Plate IX.)

It is apparent from what has been said that the micrococci, bacilli (Ewart), and fungi (Letzerich), which have been supposed to be the cause of diphtheria, present no morphological characters by which they can be distinguished from similar organisms which are found in the mouth and fauces of patients suffering from another disease in which the throat is involved — scarlet fever, and of healthy individuals — at least so far as the micrococci are concerned.

Morphological identity cannot, however, be taken as proof of physiological identity, and indeed we have ample evidence that certain organisms demonstrated to have pathogenic properties do not differ in form from others known to be harmless.

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[1] Kinnicutt in Supplement to Ziemssen's Cyclopædia, p. 82.

The elaborate and carefully conducted investigations of Wood and Formad, made under the auspices of the National Board of Health, give support to the view that the micrococcus found by them in diphtheritic exudations is the infectious agent in this disease.

In an editorial in the "Medical Times" (April 22, 1882), Dr. Wood says:—

> "A number of experiments were made upon the effect of boiling the membrane, and it was found that if the heat were maintained for only four or five minutes the contagious power was not always destroyed, but that when the boiling was continued for fifteen minutes or longer, inoculation with the virus always failed to produce any local or general effects. Culture experiments with this innocuous virus showed that the boiling had killed the micrococci, which entirely refused to grow. It is scarcely necessary to point out the confirmation this lends to the belief that the micrococci are the *materies morbi*. . . .
>
> "A number of cultures were also made, and inoculations with the liquid practised. In six or eight instances the second, third, or fifth generation of cultured plants caused the death of the rabbit. In all these cases micrococci were abundant in the blood and internal organs. In some animals the local exudations were marked, and resembled those of diphtheria; but in other rabbits the local symptoms were only slight swelling and infiltration of the surrounding tissues with serous

liquid containing an abundance of micrococci." [It must be remembered that the injection of normal human saliva into rabbits produces similar results.]

"The membrane with which these experiments were made was obtained by Dr. Formad in the neighborhood of Lakeview, Michigan. . . . The cases were not so violent, nor the contagion so marked, as in the Ludington plague, and the culture studies clearly showed that the growth power of the micrococci was correspondingly feeble.

"A very important and curious observation was made by Dr. Formad at the spot of the epidemic. The pigs of a family living in an isolated position in the forest were fed with slops from a room where three or four children were sick with the disease. Several of the pigs sickened, and one died. At the autopsy made by Dr. Formad, the larynx and respiratory passages were found entirely free from the disease, whilst the lower end of the œsophagus, the stomach, and the upper duodenum were coated with a very thick false membrane loaded with micrococci, and containing the other anatomical elements of true diphtheritic membrane. Underneath this false membrane the mucous membrane was inflamed, and in numerous places ulcerated. In the blood of the pig, as well as in the spleen and bone-marrow, the micrococci were exceedingly numerous. They were seen attacking the leucocytes, and in all other particulars conforming with the action of the plant in malignant diphtheria. Inoculation of rabbits with the membrane from the stomach of the pig produced sickness and death, with symptoms, and local and general lesions, similar to those caused by the human membrane. This observation is very important, as showing the local nature of diphtheria in its first onset, and especially as raising the suspicion that the swine-plague of the West has close relations with human diphtheria."

Several observers have noted the occurrence of an infectious disease among fowls, attended with the formation of false membrane in the trachea, which is supposed by some to be identical with diphtheria in man.

Nicati has successfully inoculated various animals with the false membrane, and states that the outbreak among fowls studied by him coincided with an increase of diphtheria among the inhabitants in the vicinity.

DISEASE PRODUCED BY BACILLI. — Abstract of a paper by J. Cossar Ewart, M. D., Professor of Natural History, University of Edinburgh: —

"About the end of March of this year, a new form of fever made its appearance in Aberdeen. The fever began with the usual symptoms; there was well-marked rigor; then a sense of coldness for some hours, accompanied with great depression; the pulse was rapid, and the temperature increased in some cases to 105° Fahr. In the worst cases there was delirium. One of the most characteristic symptoms was an affection of the deep cervical glands near the angle of the jaw; the glands enlarged; there was a feeling of fulness about the throat, congestion of the tonsils, and pain along the course of the lymphatics of the side of the neck affected. In from twenty-four to forty-eight hours the fever subsided, leaving the patient in a state of great exhaustion. In most cases there was a relapse, which corresponded exactly with the first attack, with the difference that another set of glands and lymphatics was affected.

"After this relapse, there was again apparent recovery, and then a second relapse; in some cases there were as many as six relapses occurring regularly every second day. In nearly all the cases, recovery was slow; and, in some, abscesses formed near the angle of the jaw, and in the region of the jaw. In three cases the disease proved fatal.

"When an inquiry was instituted, it was found that over three hundred individuals had suffered from this disease, and that all the sufferers had been using milk from the same dairy. A sample of milk secured for examination, when the epidemic was at its height, was found to contain numerous micrococci, spores of fungi, and spores which resembled those of *Bacillus anthracis*, — the organism which is associated with splenic fever. When cultivated, the spores germinated, first into exceedingly delicate bacilli, and then into spore-bearing filaments. On inoculating rats with the milk containing the spores, death followed in from eighteen to twenty-four hours. The tissues of the rats, especially in the region of the neck, were infiltrated with bacilli, which, on cultivation, developed into spore-bearing filaments. Inoculation proved both bacilli and spores to be as virulent as the original spores found in the milk. Confirmatory evidence of the relation of the bacillus to the disease was obtained by the examination of pus from an abscess over the angle of the jaw of one of the sufferers. This pus contained spores and bacilli similar to those found in, or developed from, the milk. Rats inoculated with a minute quantity of the pus, suffered and died in the same way as the rats infected with the milk, and the milk cultivations. Further investigations proved that the organisms had been added to the milk along with water. . . .

"Experiments, after the methods employed by Burdon-Sanderson, Pasteur, Greenfield, and Buchner, showed (1) that this bacillus could not be converted into the hay bacillus (*B. subtilis*); (2) that the cultivations became gradually less active until they were quite innocuous; (3) that, when filaments were kept for a time at a temperature which prevented the formation of spores, the virulence became attenuated, and ultimately disappeared." — British Med. Journal, Nov. 4th, 1882.

FATAL EPIDEMIC AMONG FISH CAUSED BY BACTERIA. — Dr. Ogle gives an account of an epidemic among the perch in Lake Geneva, studied by Farel and Du Plessis. The fish became sluggish, suffered from a bilious diarrhœa, and the anterior part of the head and body was injected with blood. The intestines were distended with a transparent fluid containing myriads of bacteria. The blood was diffluent, and contained "bacteria and vibrios" while the fish was still living. Experiments proved that the disease was not communicable to batrachia or to warm-blooded animals.

Professor Huxley has given an interesting account of an infectious disease of the salmon, which is apparently produced by a *Saprolegnia* identical with that which infests the bodies of dead insects.

GLANDERS. — The discovery of the parasite of glanders has recently been announced by Schutz and Loeffler, who have pursued an experimental investigation relating to the etiology of this infectious disease of the horse, in Koch's laboratory in Berlin. The parasite is said to be a bacillus resembling that of tuberculosis. It is found in the tubercles which are characteristic of the disease. The culture-medium employed was sterilized serum from the blood of the horse or of the sheep. This was inoculated with a bit cut from one of the tumors, due precautions being taken to prevent accidental contamination. The bacillus multiplied abundantly in the course of a few days. Animals of various species were inoculated with pure-cultures, and were found to differ as to susceptibility. As a rule, ulcers occurred at the point of inoculation, in rabbits, guinea-pigs, mice, etc., which had an indurated base, and the lymphatic glands in the vicinity of these were tumefied and indurated. When the dose was large, inflammation of the testicles, ovaries, and other organs, was liable to occur. Some of the animals died in the course of a few days. In these, bacilli were found which could be propagated by cultivation, but which were smaller than those found in the original material.

Two horses were inoculated successfully, and one died at the end of fourteen days. Both exhibited characteristic symptoms of the disease.

In a case of acquired glanders in man, recently studied by Wassilieff, bacilli, resembling those described by the authors quoted, were found in the nasal secretion, in blood, and in pus from pustules. They were especially abundant in the unripe pustules, and nearly all contained four to six spores.

Evidence of the inoculability of glanders from the horse to the rabbit, and from the rabbit to the ass, has also been presented by Galtier, in a communication to the French Academy of Sciences.

This author states, however, that it is not transmitted with certainty, so that the rabbit cannot be used as a test in doubtful cases, inasmuch as positive results alone are of value. In successful cases the lesions resemble those of purulent infection, and caseous deposits form at the point of inoculation. It is only exceptionally that lesions are found in the lungs and nasal mucous membrane.

GONORRHŒA. — The constant presence of micrococci in the pus of specific urethretis has now been verified by numerous observers. Neisser of Breslau is said to have first observed them, and in a paper published in 1879 he advances evidence in favor of the belief that they are the cause of the specific virulence of the fluid in which they are found. According to this author and to Weiss (1880), these micrococci are found in gonorrhœal pus from the male urethra, and in that from the female vagina, in blenorrhœa neanotorum, and in gonorrhœal ophthalmia. On the other hand, Neisser failed to find them in pus from other sources — chancres, bubo, etc. Weiss also confirms Neisser in this, and states that they are not present in the secretions of simple urethretis. Recently this subject has been investigated in a painstaking manner by Mr. A. S. Keyser (medical student in the University of Maryland). His observations fully confirm Neisser as to the constant presence of the "gonococcus" in specific purulent discharges, and its absence from non-specific pus from various sources.

Neisser claims that this micrococcus has distinct morphological characters, and describes it as follows: The micrococcus, at first round, becomes oval, and then divides transversely, forming a pair. The individual members of this pair are soon separated from each other by a slight interval, and each becomes oval and divides at right angles to the first line of division, thus forming a group of four. These groups are seen in the interior of the pus cells, and in some cases they are so numerous that the cells are completely filled with them, and resemble very closely the plasma cells which have been described by Ehrlich (see Figs. 12 and 13).

The writer is able to confirm Neisser, as to the presence of these micrococci in gonorrhœal pus, and as regards the correctness of his description of their morphological characters and mode of grouping — in pairs of oval elements and in fours as a result of transverse division in two directions. But his observations have not led him to the conclusion that these morphological characters are peculiar to the micrococcus of gonorrhœal pus (consult bibliography for titles of his papers upon this subject). Thus in Fig. 6, Plate IX., we have a photographic representation of a micrococcus of the same dimensions, and which multiplies in the same manner, which I have frequently found in normal human saliva, commonly attached to the surface of (or imbedded in the interior of?) an epithelial cell, where it forms little groups, as seen in the figure, exactly resembling those found in the cells of gonorrhœal pus. The photo-micrograph was made from a specimen obtained by cultivating the organisms found in normal saliva in an acid solution of malt extract. In my paper published in Vol. II, No. 2, of "Studies from the Biological Laboratory, Johns Hopkins University" (Bacteria in Healthy Individuals), this micrococcus was incorrectly described as a species of *Sarcina*, as "division by two perpendicular partitions in such a manner that multiplication takes place in two directions" is given as a distinctive character of this genus (see p. 96 of the present volume).

It is well known, from the observations of numerous microscopists, that pus from various sources — e. g., acute abscesses, surgical injuries, etc. — contains micrococci.

Ogsten has given much attention to the study of these, and in his report on "Micro-organisms in Surgical Diseases," he gives figures of micrococci which resemble very closely, if they are not identical with, the "*gonococci*" of Neisser. In his description of these he says: —

> "In the chain-form, division occurred in only one direction, through a plane midway between two given poles, so that a pair of cocci growing formed a chain of four; this grew into a chain of eight. . . . In the grouped form, fission took place in any direction, a single coccus seemingly dividing into two, three, or four cocci, and a continuance of this forming the groups. Many of the masses had evidently been produced by

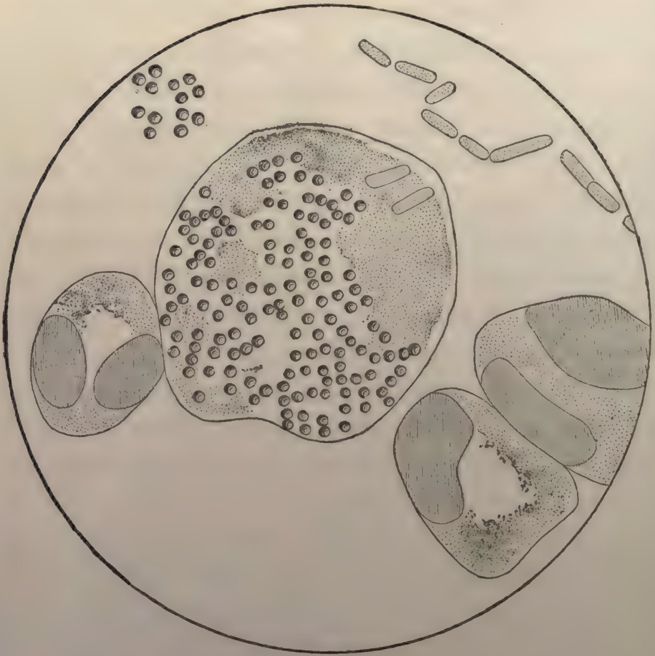

Fig. 12.

Pus cell invaded by micrococci, Ogsten. × 2600 diameters.

pairs being first formed, each of which again formed pairs, and so on. . . . In some cases, unusually large oval cocci existed, chiefly in pairs. . . . Sufficient evidence was not obtained to decide whether these different appearances indicated different species of micrococci; but the constancy with which chains produced only chains, and groups only groups, in the experiments that fall to be detailed subsequently, rather favored the suspicion of their being so."

The invasion of a pus cell by micrococci is shown in Fig. 12, which is copied from the plate accompanying Ogsten's report. When due allow-

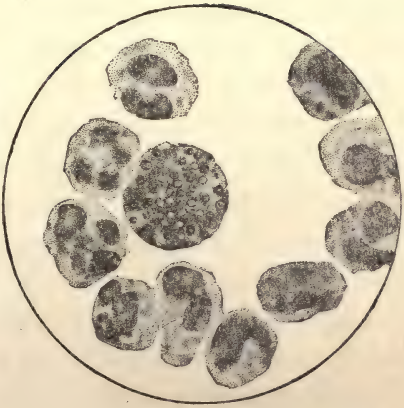

Fig. 13.

From gonorrhœal pus. Copied from a photo-micrograph. × 1000 diameters.

ance is made for the difference in amplification, it must be admitted that the resemblance is very striking to the pus cell, from gonorrhœal pus, filled with micrococci, which is seen in the centre of Fig. 13, which is copied from a photo-micrograph made by the writer.

In a recent series of experiments relating to the comparative value of disinfectants, the writer had under daily observation, for several weeks, pure cultures of the micrococcus of gonorrhœal pus, and of the micrococcus from pus contained in an acute abscess (whitlow). Many successive generations of these micro-organisms were cultivated in the little hermetically-sealed flasks described on p. 179. Culture No. 1, in one series, was obtained by inoculating the sterilized *bouillon* in such a flask with a minute drop of gonorrhœal pus at the moment of its escape from the *meatus urinarius.* In the other series, a minute drop of pus from a deep-seated abscess was used in like manner to inoculate culture No. 1 at the moment of its escape from a deep incision. No difference was detected in the morphological characters, or in the behavior in a culture-fluid, between the micrococci from these two sources. In both cases multiplication occurred sometimes in one direction, forming a linear series, — torula-form, — and sometimes in two directions, forming groups of four. Sometimes a group of three would be seen, in which one large oval micrococcus was faced by two smaller ones, which evidently had resulted from the transverse division of one member of a pair of oval elements.

My observations show that the microscopic plants under consideration *vary considerably as to size in the same culture-fluid;* and in different media they present marked differences in this respect. The individual cocci in a group, like that in Fig. 5, Plate IX. may be seen, by close inspection, to vary considerably in size. The grouping, also, depends, to some extent, at least, upon whether they are favorably situated for vigorous growth, or otherwise. When in a limited quantity of culture-fluid the pabulum required for their development is exhausted, they settle to the bottom, where they are found in little masses, or as a pulverulent precipitate; and the association into chains or groups of four is no longer observed.

The claim, then, made by Neisser, "that there is present in the purulent discharge of gonorrhœa, whether from urethra, vagina, or conjunctiva, a micrococcus not found in other pus, distinguished by its size, shape, and mode of reproduction"[1] does not seem to the writer to be sustained.

My own observations, however, agree with those of Neisser as to the constant presence of oval micrococci, mostly arranged in groups of two and four, in the pus of gonorrhœa — invading the pus cells — and I have failed to observe this arrangement in pus from other sources, although I have seen it in micrococci infesting the shed epithelium present in normal saliva. The observations of Dr. Ogsten have, however, been far more extended than my own, and he records the fact that, in a certain proportion of the specimens of pus, from acute abscesses and other sources, which he examined, this mode of grouping was seen, although the chain-form was more common. He says: —

> "In some cases, unusually large cocci existed, chiefly in pairs. For the most part these varieties existed in separate abscesses, but it frequently occurred that an abscess contained both chains and groups. Out of sixty-four abscesses where this point was specially noted, seventeen contained chains only, thirty-one groups only, and sixteen both forms, or only pairs."

In this, as in other infectious diseases, the only satisfactory evidence that the micro-organisms present in the virulent material are the infectious agents, is to be derived from inoculation experiments with a pure-culture. Unfortunately for science, but not for the animals, the lower animals commonly used in experimental studies of this nature are not susceptible to inoculations in the urethra, the vagina, or the conjunctival sac, with the most virulent gonorrhœal pus. This fact is established by the experiments of several independent observers, and has been verified by the writer as regards the dog, the rabbit, and the guinea-pig.

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[1] Belfield, in his Cartwright Lectures. The Medical Record, Vol. XXIII. No. 10, p. 253.

> "Königstein has made frequent inoculation experiments with the secretions of *blenorrhœa neonatorum*. This was smeared into the eyes of dogs and rabbits; and in some cases after so doing the eye was sewn up. Here all results were negative, even those made on puppies which were still sucking. In speaking of the microscopic examination of the secretions, Königstein confirms Neisser's discovery, *but does not agree with him in considering the diplococci as characteristic of a gonorrhœal inflammation of a mucous membrane*." (Quoted from Keyser, italics by writer.)

Eklund also finds that the "gonococci" of Neisser are uniformly present, but he decidedly rejects the opinion that they constitute in an exclusive sense the microbes of blennorrhagia, since he has discovered organisms precisely similar in cases of acute and chronic ulceration of the bowels and lungs, and also of ulcerative stomatitis. In fact, he regards these gonococci (to use his own expression) as a sort of pathological "sappers and miners." But Dr. Eklund has also discovered in pus and the superficial exudations of the inflamed urethral mucous membrane an entirely new species of parasite, which he denominates *ediophyton dictyodes*. This, like all similar microbes, is propagated by the rapid and simultaneous extension of a vast network of mycelium-filaments into the glands, the lacunæ, and the ultimate cellules of the affected structure.

It will be seen from the above that Dr. Belfield is not quite right in his assertion that "The reports have been, with one exception, unanimous in corroborating Neisser's assertion in all its details" (Cartwright Lectures, *l. c.* p. 253). Moreover, it may be questioned whether in the array of names presented there may not be some who have not given sufficient attention to the study of bacterial organisms to give much weight to their assertion that the "*gonococcus*" of Neisser presents distinct morphological characters.

Krause also found that rabbits, cats, and mice were insusceptible; but, in the case of four newborn rabbits, successful results were obtained by inoculations on the conjunctiva with material from a pure culture.

Not having the original memoir of this author at hand, the writer does not feel justified in offering an opinion as to the scientific value of the results recorded. But it must be conceded that the exactions of science demand (*a*) that rabbits of the same age be inoculated in the same manner with pus from other sources — not virulent; (*b*) that the experiment be successfully repeated; and (*c*) that the virulent nature of the inflammation produced be proved by successive inoculations on the conjunctivæ of a series of young rabbits.

In 1880 "Bokai cultivated the cocci from secretions of (*a*) an acute conjunctival blenorrhœa which was a few days old; (*b*) an acute conjunctival blenorrhœa of the second week; (*c*) acute gonorrhœa of the first, second, and third weeks. Bokai does not describe his exact method of cultivation, but contents himself with saying, that it was done in such a way as to preclude the presence of other organisms. Each of his culture-fluids *after two or three weeks* was swarming with micrococci, which were in every way identical with those described by Neisser. With these cultivated micrococci infection experiments were made on the human urethral mucous membrane. Six students, whose self-sacrifice in the interest of science is ever to be commended, offered themselves as subjects of experiment. In three cases an acute urethral gonorrhœa with all the well-known symptoms was caused." (Quoted from Keyser.)

The fact that no details are given as to the method of cultivation, and that the experimenter is not known as an expert in investigations of this kind, leaves ground for doubt as to whether pure cultures were used in this experiment; and there is also room for the ungenerous suspicion that the three victims may have contracted the disease in the usual way. Moreover, the statement that the culture-fluids were swarming with micrococci after two or three weeks is contrary to the results obtained in a large number of experiments made by the writer. In every instance the micrococci multiplied abundantly in a culture-fluid during the first twenty-four hours after it was inoculated with gonorrhœal pus. But after forty-eight hours all development ceased, in consequence of the pabulum being exhausted, and the micrococci fell to the bottom of the flask.

"In September, 1882, Bockhart published his experiment in inoculating the gonococci on the sound human urethral mucous membrane. His description leaves nothing to be desired in point of clearness. The subject of the experiment was a forty-six-year-old paralytic, completely anæsthetic, whose death was expected daily. The material used for infection consisted of gonococci grown in fresh infusion of gelatine through four generations.

"The urethra of the person experimented upon was previously perfectly sound. Forty-eight hours after injection there appeared at the meatus urinarius a slight redness, and on pressure a small quantity of mucous secretion could be obtained. The symptoms increased, and on the sixth day a typical gonorrhœa was formed, which increased in severity up to the twelfth day, when the man died. During the whole time the characteristic gonococci were found in the abundant secretions." (Quoted from Keyser.)

The criticism which the writer feels called upon to make in this case, which is thought by Keyser to be very convincing, is that a series of four successive cultures is not sufficient to insure the exclusion of the original material *when the cultivation is conducted upon a solid substratum.* As multiplication only occurs upon the surface of the culture-medium, the material used to inoculate culture No. 1 is not diluted in a series of cultures, as in the method described on page 238, and we have no longer the astonishing array of figures there given to show the practical exclusion of a hypothetical, non-living virus. When we consider that material from the surface of culture No. 1 is transferred to the surface of culture No. 2, and so on, we must admit the possibility that some of the original material may have been transferred to culture No. 4.

This source of error was excluded in the following experiments:—

"The pus, from which the cultures used in these experiments were started, was taken from cases [of gonorrhœa in the male] in the acute stage of the disease, and which had not been subjected to any local treatment.

"*Exp. No.* 4 (July, 1882).—Made by Dr. Hirschfelder with material furnished by the writer. A culture fluid, fifteenth, containing the micrococcus of gonorrhœal pus, was introduced into the urethræ of three patients in the city and county hospital, upon small wads of cotton which were thoroughly moistened with the fluid, and left *in situ* for fifteen minutes.

"*Case* 1.—J. D. has been in bed for about nine months; caries of the vertebræ.

"*Case* 2.—J. B., colored; syphilitic paralysis.

"*Case* 3.—D. M., in bed some time; aneurism of the abdominal aorta. *The result was entirely negative.*

"*Exp. No.* 5 (August, 1882).—A fresh culture, fourteenth, from another, and recent, case was introduced in the same manner into the urethra of J. D., subject of previous experiment. *Result negative.*

"*Exp. No.* 6 (August, 1883).—A fresh culture, thirteenth, was introduced into the urethra of W. B. *Result negative.* . . .

"*Exp. No.* 15 (October 5th, 1882).—A pure culture of the micrococcus of gonorrhœal pus (the thirtieth culture, or above), was introduced into the urethræ of two healthy men [G. M. S. and V. D.], by means of pledgets of cotton wool soaked in the fluid, which were left *in situ* for fifteen minutes. *Result entirely negative.*"

A somewhat extended account has been given of these experiments relating to the etiology of gonorrhœa, because it is deemed a matter of great scientific importance to determine, in a definite manner, the relation of the micrococcus, demonstrated to be constantly present, to the infective virulence of the material containing it. It is evident that if a single infectious disease is shown to be independent of all micro-organisms, no generalization in favor of the parasitic-germ theory will be possible, and the etiology of each infectious disease must be worked out separately by the experimental method.

In the disease under consideration, it is evident that the contradictory results reported call for further investigation; and, notwithstanding the negative results which have attended his own experimental inoculations, and the fact that the "*gonococcus*" of Neisser has not distinctive morphological characters, the writer will be very ready to admit the essential etiological *rôle* of this micrococcus whenever it is demonstrated that a pure culture introduced into the urethra of man, or into the conjunctival sac of young rabbits, is followed by a specific inflammation, as shown by the virulent character of the purulent discharge which attends it.

HYDROPHOBIA. — That illustrious men are not always infallible, is shown by the error into which Pasteur fell in ascribing to a micrococcus commonly found in human saliva, the power of producing hydrophobia. The experiments which led to this conclusion, which was communicated to the French Academy in 1881,[1] were made with the saliva of a child, five years of age, which died from hydrophobia in one of the hospitals of Paris, December 11th, 1880. This child had been bitten in the face, a month previously, by a mad dog. Four hours after death, a little buccal mucus, gathered by means of a brush, was injected into two rabbits. These rabbits were found dead December 13th. Other rabbits were inoculated with the blood of these, and death occurred even more rapidly. Successive inoculations, repeated many times, gave the same result. The rabbits showed at the autopsy the same lesions. (These will be described in the account given of induced septicæmia in the rabbit, p. 359.)

According to Pasteur, death is produced by the injection of blood or of saliva, and the blood of the animal inoculated contains a microscopic organism having very curious properties. Dogs inoculated with the "new disease" fall sick immediately, and usually die in a few days, without manifesting any of the true symptoms of hydrophobia. Rabbits inoculated from mad dogs have a variable period of incubation, so that the disease in question cannot be identical with hydrophobia. Pasteur, then, did not commit the error of describing this "new disease" as hydrophobia, but he made the erroneous assumption that the saliva of the child was virulent because it had died of hydrophobia, whereas the writer has shown that the same infectious disease results from the injection into the subcutaneous connective tissue of rabbits of normal human saliva. This fact was first disclosed by an experimental injection of 0.5 c.c. of my own saliva, made in New Orleans, La., September 29th, 1880, nearly three months prior to the death of the child from which Pasteur obtained saliva for his experiments; and my first experiments in New Orleans were followed by many others made in Philadelphia during the month of January, 1881, and in Baltimore during the months of June and July of the same year.

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[1] *Comptes rendus*, XCII. p. 159.

Pasteur soon became convinced that the microbe of his "new disease" had nothing to do with hydrophobia, and he has recently[1] communicated additional facts of the greatest importance bearing upon the etiology of this disease. These facts he has summarized as follows:—

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[1] *Comptes rendus*, XCV. pp. 1187–1192.

"I. The silent (*la rage mue*) and furious forms of rabies proceed from the same virus. Indeed, we have found experimentally that one form may give rise to the other.

"II. Nothing is more varied than the symptoms of rabies. Each case has, so to speak, its own peculiar symptoms, the special characters of which, there is reason to believe, depend upon the particular part of the nervous system, of the brain, or of the spinal marrow, where the virus locates itself and multiplies.

"III. The virus is associated in the saliva of a rabid animal with various microbes; and this saliva may cause death, by inoculation, in three different ways:—

"By the new microbe which we have made known under the name of the *microbe of saliva;*

"By the excessive development of pus;

"By rabies.

"IV. The medulla oblongata of a person, or of one of the lower animals, dead from rabies, is always virulent.

"V. The virus of rabies is not only found in the medulla oblongata, but also in the entire brain or a portion thereof.

"It is also found localized in the spinal marrow, and often in every portion of it.

"The virulence of the spinal marrow is quite equal to that of the medulla oblongata or of the brain; and this is true of the inferior as well as of the superior portions.

"So long as the brain and spinal marrow are not invaded by putrefaction this virulence persists. At a temperature of about 12° C. we have been able to preserve the virulence of the brain of a rabid animal for three weeks.

"VI. In order to produce rabies with certainty and rapidity, it is necessary to inoculate the surface of the brain, in the cavity of the arachnoid, by means of trephining.

"The same result is obtained by introducing the virus directly into the blood.

"These methods of inoculation frequently give rise to the disease at the end of six, eight, or ten days.

"VII. Rabies communicated by introducing the virus into the blood very often presents characters quite different from those of furious rabies, resulting from a bite or from inoculation upon the surface of the brain, and it is probable that many cases of silent rabies have escaped observation. In the cases which may be denominated *medullary*, prompt paralyses are frequent, furor is often absent, and the rabid barkings are rare; on the contrary, the itching is sometimes terrible.

"The details of our experiments lead us to believe that, in the method by intravenous injection, the spinal marrow is first attacked; that is to say, that the virus first fixes itself and multiplies in this locality."

INTERMITTENT FEVER. — The limits of the present volume do not admit of an extended account of the experimental evidence which has been advanced in favor of the parasitic-germ theory as regards the etiology of the malarial fevers. The fact that the malarial poison is evolved under circumstances which favor the development of low organisms, and that its production has been pretty definitely proved to be associated with the decomposition of organic material of vegetable origin—which has been proved to depend upon the presence of bacterial organisms, has led many physicians confidently to anticipate that a malarial germ would be found in the bodies of those suffering from malarial poisoning; and since the demonstration of the anthrax bacillus, and the spirillum of relapsing fever, it seems to have been rather hastily assumed that all disease germs are to be sought especially in the blood. In intermittent fever, however, it would seem, *a priori*, that the hypothetical parasite would not be likely to find a suitable culture-medium in the blood of a living animal; (*a*) because its normal habitat is in swamps, where its development is associated with the decomposition of vegetable matter; and (*b*) because, so far as we know, parasitic micro-organisms, which multiply freely in the blood of living animals, produce infectious diseases communicable from one individual to another, whereas we have no evidence that this ever occurs in the paludal fevers. Conditions more nearly approaching those which favor the development of the poison external to the body may, however, be found in the alimentary canal, and we may suppose that the germ locates itself here. Or we may admit the possibility that its action is restricted to the production of a volatile chemical poison which is evolved as a result of its vital activity in the localities where it abounds external to the body; and that this infects the atmosphere in the vicinity, and produces malarial poisoning in those who respire this atmosphere. But this is speculation, and cannot stand before the results of exact experiments. Let us then briefly review these results, or at least those which have been most recently reported, and seem most worthy of attention.

Passing by the researches of Salisbury and other claimants to the honor of having discovered the malarial parasite, we come at once to the investigations of Klebs and Tommasi-Crudeli, made in the vicinity of Rome, and as a result of which they announced in 1879 the discovery of the *Bacillus malariæ*.

The evidence in favor of this discovery is stated so concisely in an editorial in "The Medical News"[1] that the writer takes the liberty of quoting from this for his present purpose:—

> "These observers found in the earth of malarial districts, in Italy, numerous shining oval and mobile spores, .95 of a micro-millimetre in the longer diameter. They were able to cultivate these spores in the animal body as well as in culture experiments, and the animals infected by them exhibited not only the clinical course of malarial disease as seen in man, but also the *post mortem* appearances; while the bacillus was also found in the blood of such animals, taken after death. The spores develop in the animal body, as well as in culture-experiments, into long threads, which are at first homogeneous, but later divide, while new spores develop in the interior of the segments. The position of the spores, which are

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[1] Philadelphia, January 13th, 1883, Vol. XLII. No. 2, p. 41.

found either at the poles, or in the middle of the segment, serves as a mark of distinction between this and other pathological bacilli.

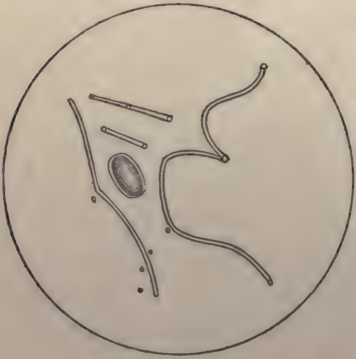

Fig. 14.

Bacillus malariæ in blood drawn during life from the spleen of a person suffering from malarial fever. (From Cuboni and Marchiafava, in Klebs' Archiv, etc., 1881.) *N. B.* These bacilli were found in one case only.

"Following Klebs and Tommasi-Crudeli, Marchiafava and Cuboni, in Italy,[1] studied the blood of men ill with malaria. In this they found spores and bacilli which they declared to be identical with those described by the former. The spores included in the white blood-corpuscles were sometimes so numerous as to seem to fill them completely. Similar studies on malarial patients by Lanzi, and again by Peroncito, led to the same conclusions.

"Succeeding these, Marchand published in Virchow's 'Archiv'[2] some observations really made in 1876, whence he concluded that there exists in the blood, in the cold stage of intermittent fever, mobile and flexible rods, presenting slight swellings at their ends, and sometimes also at the middle. These end-swellings he thought also might be of the nature of spores.

"More recent still are the elaborate experiments of Professor Ceri, of Camerino, Italy, published in the 'Archiv für experimentelle Pathologie.'[3] These consisted of culture-experiments with organisms found in malarial and other soils, of experiments on animals, and culture-experiments with quinine. They resulted in proving that the spores could be cultivated, — Ceri applying the term *natural germs* to those found in the atmosphere and soil, and *artificial germs* to those which result from their culture; that animals could be infected by their injection into the blood, though to a less degree by the cultivated than by the natural germs, the former growing weaker in successive generations; and that the infecting properties could be retarded by the application of heat to culture-fluids, and the introduction of quinine into them, certain degrees of the former and strengths (1: 800) of the latter making the culture of the spores impossible, and arresting the putrid fermentation induced by them. The practical application of these facts is self-evident.

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[1] Archiv für experimentelle Pathologie, Vol. XIII.

[2] Vol. LXXXVIII. p. 104, April, 1882.

[3] Vols. XV. and XVI. 1882.

"Finally the opportunity has recently been presented to Dr. Franz Ziehl to test these results clinically[1] in three typical cases of malaria, in all of which the spleen was enlarged. In all three the bacilli above described were found in the blood taken from any part of the body by the prick of a needle, and examined in the fresh state, or dried in a thin layer upon a cover-glass, by simply passing the latter over a flame. These have been preserved by Dr. Ziehl for three months without undergoing any change.

"The bacilli thus observed were of different lengths, but usually were from one-fourth to the entire diameter of a red corpuscle. The majority of those measured were about 4 micro-millimetres long and .7 broad. Their ends were swollen and roundish."

The evidence as here stated certainly seems very

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[1] Deutsche medizinische Wochenschrift, Nov. 25, 1882.

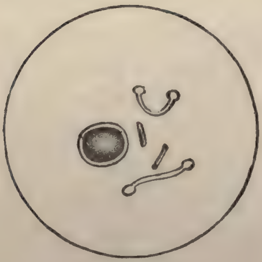

Fig. 15.

The *Bacillus malariæ* as seen in blood obtained from a patient seized with intermittent fever, taken during the chill. (From Tommasi-Crudeli.)

complete, and the writer freely admits that the negative results which he reported after an attempt to repeat the experiments of Klebs and Tommasi-Crudeli, made under the auspices of the National Board of Health in New Orleans, during the summer of 1880, cannot be given great weight as opposed to these positive statements. But the fact that no confirmation has yet come from English or American sources during the time which has elapsed since the discovery of Klebs and Tommasi-Crudeli was announced, constitutes negative evidence of a much stronger character. The *Bacillus malariæ*, according to all accounts, should be much easier to recognize than Koch's bacillus of tuberculosis, which has already been seen by numerous physicians in nearly every large city in this country and in Europe. But who on this side of the Atlantic has seen the *Bacillus malariæ?* Yet malarial fevers are widespread, and a microscope is to be found in nearly every physician's office. The writer has searched faithfully for this bacillus in the blood of patients in the Charity Hospital, New Orleans, selected for him by Professor Bemiss as well-marked cases of malarial fever, but admits that he has not sought it in blood from the spleen, or in that drawn during a chill, except in two or three instances. He is therefore anxious to make more extended researches whenever the opportunity may offer, and will not fail to report promptly any future observations more in correspondence with those of the German and Italian investigators named.

In the report of the experimental investigation referred to, the following summary statement is made: —

> "Among the organisms found upon the surface of swamp-mud, near New Orleans, and in the gutters within the city limits, are some which closely resemble, and perhaps are identical with, the *Bacillus malariæ* of Klebs and Tommasi-Crudeli; but there is no satisfactory evidence that these or any of the other bacterial organisms found in such situations, when injected beneath the skin of a rabbit, give rise to a malarial fever corresponding with the ordinary paludal fevers to which man is subject.
>
> "The evidence upon which Klebs and Tommasi-Crudeli have based their claim of the discovery of a *Bacillus malariæ* cannot be accepted as sufficient; (*a*) because in their experiments and in my own the temperature curve in the rabbits experimented upon has in no case exhibited a marked and distinctive paroxysmal character; (*b*) because healthy rabbits sometimes exhibit diurnal variations of temperature (resulting apparently from changes in the external temperature) as marked as those shown in their charts; (*c*) because changes in the spleen such as they describe are not evidence of death from malarial fever, inasmuch as similar changes occur in the spleens of rabbits dead from septicæmia produced by the subcutaneous injection of human saliva;

(*d*) because the presence of dark-colored pigment in the spleen of a rabbit cannot be taken as evidence of death from malarial fever, inasmuch as this is frequently found in the spleens of septicæmic rabbits.

"While, however, the evidence upon which Klebs and Tommasi-Crudeli have based their claim to a discovery is not satisfactory, and their conclusions are shown not to be well founded, there is nothing in my researches to indicate that the so-called *Bacillus malariæ*, or some other of the minute organisms associated with it, is not the active agent in the causation of malarial fevers in man. On the other hand, there are many circumstances in favor of the hypothesis that the etiology of these fevers is connected, directly or indirectly, with the presence of these organisms or their germs in the air and water of malarial localities."

It will be seen that I am not able to agree with the editorial above quoted in the statement, that "the animals infected by them"—*i. e.*, the spores of *Bacillus malariæ*—"exhibited not only the clinical course of malarial disease as seen in man, but also the *post mortem* appearances." On the other hand, I do not find in the temperature-charts published by Klebs and Tommasi-Crudeli in their original report, and copied by me in my report referred to, satisfactory evidence of the production of a fever characterized by regularly recurring paroxysms, like the ordinary paludal fevers in man; nor do I consider the *post mortem* appearances sufficiently characteristic to warrant the inference that these animals died of a fever identical with the malarial fevers to which the human race is so subject; especially in view of the fact, that infection did not occur in the natural way, that the rabbit is very subject to various forms of septicæmia, and that prior to these experiments no evidence had ever been presented to show that the rabbit experiences any harm from respiring an atmosphere charged with malaria.

Professor Ceri, however, claims to have produced in rabbits intense febrile paroxysms *of a decidedly intermittent type, and continuing for a long period,* by the hypodermic injection of artificially cultured malarial soil exposed for ten days to a temperature of 35° to 40° C.

This is a very definite statement, and, if supported by temperature-charts showing the fact, would have great weight.

In a recent report (March 18, 1883) to the Italian Minister of Agriculture, Tommasi-Crudeli refers to the production of intermittent (?) fevers in the lower animals by the subcutaneous injection of the blood of malarial-fever patients, and states that he made extensive preparations to continue his experiments in this direction during the year 1882; but he was unable to carry out his intention for the reason that not a single case of *pernicious* fever was received during that period into the Roman hospitals.[1]

Here, then, we have a confession which makes it evident that the *pernicious fever,* ascribed to malaria, by the author referred to, differs from ordinary malarial fevers — intermittents and remittents — which also prevail in Italy, in the essential particular that it is an infectious disease, and may be transmitted to the lower animals; as well as in the fact that it is a continued rather than a paroxysmal fever.

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[1] Quoted from a paper in the Med. Record of August 18, 1883, by Dr. C. P. Russell.

The writer has long suspected that the continued pernicious fevers of the Roman Campagna, and of other parts of Italy, differ essentially from the ordinary intermittents and remittents of this country, and that, while there is undoubtedly a malarial element, in a certain proportion of the cases at least, there is another etiological factor to which the continued and pernicious form of development manifested by the morbid phenomena must be ascribed. We know that malaria may be associated with the specific poisons of typhoid and of yellow fevers in such a way as to produce atypical forms of these diseases, and it seems not improbable that the Roman fever is in truth one of these mixed or hybrid forms of disease. In this case the bacillus of Klebs and Tommasi-Crudeli, if it has any etiological import, is probably the factor to which the continued and pernicious form of this fever must be ascribed, rather than the malarial germ, which the authors named had undertaken to discover.

Professor Ceri's experiments relating to the germicide power of quinine are extremely important and interesting. But it is well to remember that, if a dose of ten grains passed at once into the blood of an adult weighing one hundred and sixty pounds, the proportion which it would bear to the whole mass of blood in the body (estimated at twenty pounds) would be only 1:11,520; whereas Professor Ceri's experiments lead him to the conclusion, "that the muriate of quinine in the proportion of 1 : 800 prevents the development of any infectious germs."[1]

The preventive power for the *Bacillus malariæ*, however, was found to be greater than this, and in a series of eighteen experiments in which culture-solutions were infected "with a drop of blood [containing the *Bacillus malariæ*] of a rabbit into which had been injected cultures of malarial soil, the development continued absent up to 1 : 2,000, and at 1 : 2,250 it was aseptic. The *Bacillus malariæ* did not develop in the fertile cultures, which contained only vibriones."

The writer is not disposed to underestimate the value of these researches, but in a spirit of scientific conservatism would remark as follows : —

First. — Fowler's solution of arsenic also cures intermittent fever; and the germicide power of this remedy is practically *nil*, as determined by the writer in a series of experiments in which the micrococcus of pus served as a test-organism. In the proportion of forty per cent it failed to kill this organism. Its power of restricting the development of the *Bacillus malariæ* should be tested as a check on the conclusions which may be too hastily drawn from Professor Ceri's experiments with quinine.

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[1] Quoted from translation by Hugo Engel in "The Medical Times," Philadelphia, Dec. 16, 1882, p. 198.

Second. — It is not impossible that the *pernicious* malarial fevers of Italy may differ essentially from the ordinary remittents and intermittents of this country, and that their continued form is due to a septic complication, which may result from invasion of the blood by a pathogenic organism peculiar to that country or to the tropical and semi-tropical regions where pernicious fevers are most prevalent.

Third. — Fat-granules are found in the white corpuscles of the blood of yellow fever, — which disease resembles the pernicious malarial fevers in many particulars, — which bear so strong a resemblance to the spores of bacilli that a mistake might easily be made.[1] (See p. 425.) Several of the observers named found spores "included in the white blood-corpuscles, which were sometimes so numerous as to seem to fill them completely."

Fourth. — No great significance can be attached to the finding of bacterial organisms *post mortem* in the blood and tissues, especially in warm climates, unless the examination is made *immediately* after death. And even then we must admit the possibility that such organisms may migrate from the intestine, where they are always present in abundance, during the last hours of life, when the circulation is feeble, and the vital resistance of the cells intervening between the lumen of the intestine and of its capillary vessels is very feeble, or quite lost.

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[1] Compare Fig. 3, Plate XII. and Fig. 3, Plate VIII.

Finally. — The writer's observations lead him to be suspicious as regards the pathogenic *rôle* of organisms in the blood, which are few in number and require diligent search for their demonstration. And the possibilities of accidental contamination are so great, when a drop of blood is drawn from the body of a patient with the greatest possible precautions, that the finding of a rod or of a sphere supposed to be a bacillus or a micrococcus requires verification by the finding of at least several more rods or spheres of the same kind in the same specimen; and by the use of staining reagents and the test of cultivation.

A more recent discovery than the *Bacillus malariæ* of Klebs and Tommasi-Crudeli is the *Oscillaria malariæ* of Laveran (1881). This discovery is also confirmed by Richard. The first-named author says: —

"There exist in the blood of patients attacked with malarial fever pigmented parasitic elements, which present themselves under three principal aspects. . . .

"The parasitic elements are only found in the blood of patients sick with malarial fever, and they disappear when quinine is administered.

"They are of the same nature as the pigmented bodies which exist in great numbers in the vessels and organs of patients dead with pernicious fever, and which have heretofore been described as melanotic leucocytes."

These parasites are described as being somewhat smaller than the leucocytes of the blood, as sometimes resting and sometimes exhibiting amœboid movements, and as sometimes having three or four long, motile filaments attached, which are very difficult to see except when they are in motion. They contain pigment granules, commonly arranged in a circle. Richard states that the malarial parasite of Laveran invades the red blood-corpuscles, where it is first seen as a minute round spot upon the circumference. In other corpuscles it is larger, and about the margin is seen a circle of black nodules. In others still the parasite has reached such a size that only a narrow, transparent zone remains between its circumference and the cell-wall of the red corpuscle, and no trace of the hæmoglobin remains. The oscillaria is, however, still surrounded by a ring of black nodules. The parasite now escapes into the blood-serum. The motile filaments described by Laveran are also referred to by Richard, who states that in some cases they alone perforate the cell-wall of the red corpuscle, which is moved about in a peculiar manner by their oscillations.

The presence of these parasites was demonstrated in the blood of every case of malarial fever observed by Richard, and frequently they were very numerous.

We shall not attempt to estimate the scientific value of these observations, but would remark that Laveran and Richard, in their researches, seem not to have encountered the *Bacillus malariæ*, although the announcement of its discovery had been made two years prior to the date of their investigations, and should have prepared them to find it if present in the blood of malarial cases studied by them.

Leprosy. — In 1879 Hansen, in a report to the Medical Society of Christiania (Norway), stated that he had "often, indeed generally, found, when seeking for them in leprous tubercles, small, rod-shaped bodies in the cells of the swelling." These rods were not found, however, in blood recently taken from leprous patients. Certain brown cells were also described in this report as peculiar to leprosy. In a later communication (1880) Hansen says: —

"I have by this preparation [staining with methyl-violet] obtained confirmation of my earlier supposition, that the large brown bodies, after all, are nothing else than either masses of zooglœa, or collections of bacilli which are enclosed in cells. By looking at Fig. 4 [Fig. 16], which represents tumor-cells treated with osmic acid, drawn from preparations made in 1873, one is easily able to form an idea how these same cells, by a constantly increasing number of small rods, at last become quite overloaded, and thus obtain the appearance of being filled with fine granules, since the single rods cannot then be distinguished. . . .

"Since writing the above I have also been so fortunate as to obtain bacilli, finely colored, in a section of a tubercle hardened in absolute alcohol. . . . Bacilli

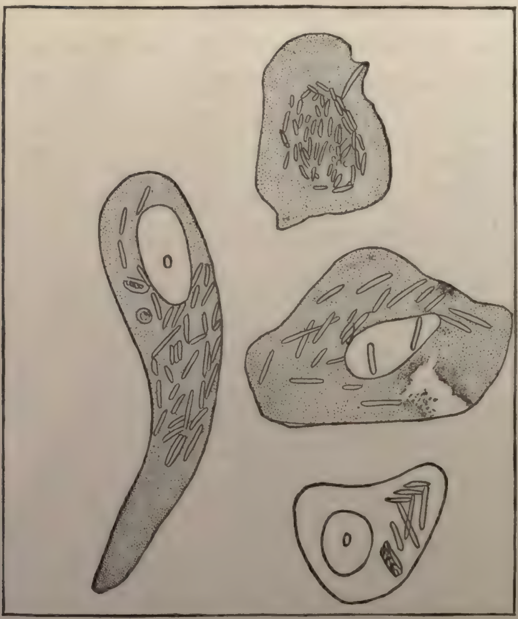

Fig. 16.

Cells from leprous tubercle containing the *Bacillus lepræ*. Copied from plate illustrating Hansen's paper in Quart. J. Micr. Sci., Jan. 1880.

are found in all parts of the section, either singly, or more frequently in groups, fully corresponding to those occurring in the cells. I furnish a drawing of two groups taken with Zeiss's immersion system $\frac{1}{12}$ and eye-piece No. 4." (See Fig. 17.)

One observer, Köber, claims[1] to have found the bacillus of Hansen in the blood of leprous patients; while Edlund ascribes the disease to a micrococcus which he finds in the blood, as well as in the leprous tubercles. Neisser, also, says that micrococci are always present in the epidermis, although he confirms Hansen as to the presence of bacilli in the leprous tubercles, *and also in the liver, spleen, testicles, lymph-glands, and other parts.* (*Query:* How much time had elapsed between the death of the patients and the autopsies.)

According to Neisser:—

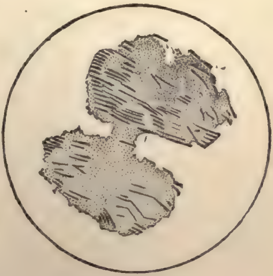

Fig. 17.

Copied from Hansen's paper above cited.

"The bacilli have the form of small, slender rods, with a length about half the diameter of a red blood-corpuscle, and about four times as long as broad. They approach most nearly the bacilli connected with the septicæmia of the mouse, but are not so fine." [According to Koch, they very closely resemble the bacillus of tuberculosis.] "They are invisible in uncolored sections, but beautifully seen when tinctured with fuchsin and gentian-violet. Their relative position and distribution vary greatly, according to the part where they are found. They lie either two or three behind one another, apparently forming a long, sometimes curved, thread; or six or seven lie parallel to one another; or large numbers are associated in all directions into a confused mass, which is only with difficulty resolved into its elements. At a later stage of the leprosy the rods break up into granules; but whether these are the result of disintegration, or must be regarded as spores, is doubtful. The bacilli were found in greatest quantities in the skin; next to that, in the testicles; also in the spleen and liver; they were not found in the marginal parts of the lymph-canals; the kidneys were free from them."[1]

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[1] Quoted from Journal of the Royal Micr. Society, Ser. II. Vol. I. Part 2, December, 1881, p. 928.

The presence of these bacilli in leprous tubercles, etc., has been confirmed by several observers in addition to those mentioned. Recently Dr. Thin, an experienced microscopist and mycologist, has reported that he finds, in the skin of Chinese lepers, a bacillus of the size and form, and same staining qualities, as that described by Hansen.[1] It is said that the bacillus is not found in the anæsthetic form of the disease.

The writer examined the blood of lepers in the Charity Hospital, New Orleans, during the summer of 1880, with a negative result, so far as the direct examination was concerned. But in culture-cells in which a drop of blood, protected from the external air, was supplied with oxygen from a small air-space, hermetically enclosed, micrococci developed, which may be seen in the heliotype reproduction of a photo-micrograph made from such a specimen, Plate II. Fig. 2.

Inasmuch as these lepers had upon the face and hands ulcerated tubercles, the pus from which was doubtless infested with micrococci, very little importance was attached to the fact that micrococci made their appearance in these culture-cells. For the chances of accidental contamination, of a drop of blood drawn from the finger, by micrococci from the surface of the body, were so great as to give but little value to the culture-experiment, notwithstanding the fact that the precaution was taken to wash the finger with alcohol before making the puncture. The writer's own experiments have since shown that this precaution is probably inadequate; for the micrococcus of pus is not killed by exposure for two hours to 25 per cent alcohol.

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[1] British Medical Journal, Aug. 5, 1882, p. 231.

Up to the present time, the supposition that the bacillus of Hansen bears a causal relation to leprosy depends for its support entirely upon the fact that it is found in the leprous tubercles, etc. It is not well established that these bacilli have distinctive morphological characters and staining reactions. Indeed, Koch finds that they closely resemble his bacillus of tuberculosis in both these particulars. But even if this bacillus were proved to be peculiar to leprosy, in the absence of successful inoculations with pure cultures its causal relation to the disease must remain in question; for, in view of what we know of the habits of the bacteria generally, there is nothing improbable in the supposition that this particular species is able to invade tissues of a low grade of vitality, and finds in the leprous tubercles the pabulum necessary for its development. If, however, leprosy is truly an infectious disease, which seems to be a matter of considerable doubt, the rapidly accumulating evidence in favor of the parasitic-germ theory, in explanation of the etiology of these diseases, lends strong probability to the first-mentioned hypothesis.

Hansen has endeavored to inoculate rabbits with leprosy, by introducing portions of the leprous growths, especially the tubercles, under the skin of these animals. He says, "I was not lucky in any of these attempts." (1880.) More recently he has inoculated a monkey, which, at the date of his report, had been under observation for six months, without having developed any symptoms of the disease. But as the time of incubation in man is said to be a year or more, this experiment is not considered decisive.

The bacilli have been successfully cultivated by their discoverer upon gelatinized blood-serum. In these cultures development commenced after an interval of three or four days, and the bacilli often presented nodular enlargements at the extremities, which were believed to be due to the formation of spores. In these cultures filaments formed, made up of a number of bacilli, and these were often so abundant as to form an entangled net-work. The fact that these bacilli multiply and develop spores in a culture-solution, within a few days, while the period of incubation in leprosy is "at least a year," seems a little difficult to reconcile with the supposed etiological *rôle* of these parasites.

MALIGNANT ŒDEMA. — According to Koch, a frequent source of error in experiments on anthrax arises from accidental contamination of the culture-fluids by a bacillus which closely resembles *B. anthracis.* This organism is called the bacillus of malignant œdema, and the disease to which it gives rise has been especially studied by Gaffky, who states that the organism is apparently identical with the *vibrion septique* of Pasteur.

Although very similar to the anthrax bacillus, Koch points out certain morphological characters which distinguish the one from the other. The anthrax bacilli are a little broader than the others, and the joints have concave extremities; whereas the others are rounded at the extremity. *B. anthracis* is motionless, while that of malignant œdema is usually in active motion. According to Ewart, the anthrax bacillus, also, is motile during certain stages in its life-history: —

> "The disease is readily produced by the introduction of a small quantity of garden-earth under the skin of an animal (rabbit, guinea-pig, or mouse). The animals become ill very soon, there being no distinct incubation period, and death occurs after twenty-four to forty-eight hours. Spreading from the point of infection, the subcutaneous cellular tissue and the intermuscular cellular tissue become œdematous and reddened, the spleen is enlarged, soft, and of a dark reddish-blue color; but the other organs are not altered to the naked eye. No bacilli, or only very few, are found in the blood of the heart immediately after death; but the fluid obtained after section of the various organs contains numbers of these moving rods. The longer the time which has elapsed after death, the more numerous do the bacilli in the tissues and blood become. They grow best in the dead body, thus differing from other pathogenic organisms. On section of the organs, the bacilli are found in the cellular tissue, almost exclusively towards the sur-

face; they apparently spread into the organs from the cellular tissue around. They may also form plugs in the capillaries, though this is rare. In some cases putrefaction occurs rapidly, but in others it is apparently retarded.

"With regard to the cultivation of these organisms outside the body, it has been found by Pasteur, Joubert, etc., that they will not develop in presence of oxygen, but readily grow when carbonic-acid gas is substituted for oxygen in the cultivating flasks. This observation is confirmed by Gaffky, who grew them in the interior of potatoes removed from the air. These bacilli caused death when injected into the subcutaneous cellular tissue, thus showing that they were the true *materies morbi.*

"Of great interest is the question of the relation of these organisms to those found by Lewis in the blood of asphyxiated animals, especially of rats, — an observation confirmed by Gaffky. These organisms are found most frequently in the blood of horses, and Koch explains this by the slower cooling of their bodies. In smaller animals, these organisms, which probably come from the intestine, do not develop rapidly, unless the body be kept at a temperature of about 38° C. Dr. Gaffky asphyxiated a guinea-pig, and then placed the body in an incubator. In twenty-four hours the body was much swollen from gas-development; and from the natural orifices bloody fluid exuded, containing numerous bacilli indistinguishable from œdema bacilli. Everywhere throughout the body, more especially in the subcutaneous cellular tissue, these bacilli were present in large numbers. A drop of fluid from the cellular tissue was injected into a second guinea-pig. This animal died on the following day, with the typical appearances of malignant œdema. A minute quantity of the œdematous fluid, diluted with distilled water, and injected into a third guinea-pig, was followed by the same result."[1]

MILK SICKNESS. — This is an infectious disease which prevails in certain rural districts in the United States, and which is said gradually to recede before the advance of improved agriculture: —

"In its source, in unimproved marshy localities, it closely resembles the malignant anthrax; also, in its communicability to all animals; but it differs essentially in that it fails to show local anthrax lesions, in place of which it expends its energy on the nerve centres, producing great hebetude and loss of muscular power. According to Dr. Phillips it is characterized by the presence in the blood of a microzyme (*spirillum*) like that seen in relapsing fever. The germ is probably derived from drinking-water, or the surfaces of vegetables, as certain wells are found to infect with certainty, and the disease has been repeatedly produced by feeding upon particular plants (*Rhus toxicodendron*, etc.). That these plants, in themselves, are not the pathogenic elements, is shown by their innocuous properties when grown in places out of the region of the milk-sickness infection. It seems altogether probable that here, as in malignant anthrax, we are dealing with a microzyme which has developed pathogenic properties, and which can be reproduced indefinitely in the bodies of living animals. The great danger of this affection consists in the conveyance of the germ with unimpaired potency through the flesh and milk, and through manufactured products of the latter, — butter and cheese.

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[1] Quoted from The British Medical Journal, July 15, 1882, p. 99.

Some even hold that in animals giving milk the system does not suffer materially, but that it is saved by the drainage of the germs through the mammary glands, and that thus a milk-sick cow may remain for a considerable time unsuspected. . . . The disorder proves fatal in man as in animals."[1]

A careful study of this disease by the experimental method would probably demonstrate its parasitic nature; and it is extremely desirable that its etiology may be worked out, both in the interest of science and of medicine.

MEASLES. — Coze and Feltz state that bacteria are found in the blood of measles, of extreme minuteness and great mobility. In the period of invasion the nasal mucus contains small "bacteriform elements." The inoculation of this blood did not produce the death of rabbits; but these animals were sick for two or three days, as the result of such inoculations, and "very slender and active rods" were found in their blood (Magnin). Klebs, also, found micrococci in the trachea, and in blood taken from the heart of infants that had fallen victims to this disease. In the blood, preserved in capillary tubes, these micrococci developed in spherical masses.

Braidwood and Vacher describe certain small spherical bodies first found by them in the breath of children in the acute stage of the disease, which they believe to be the contagious elements. These are "sparkling, colorless bodies, something like those found in vaccine, but larger." Some were spherical, others were elongated, with sharpened ends. The breath of healthy children did not contain these sparkling bodies. These bodies were also found in the lungs and liver of two children who died of measles.

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[1] Prof. James Law, National Board of Health Bulletin, Vol. II. No. 4, p. 456.

Keating has recently (1882) reported the finding of micrococci in the blood of malignant measles, and their absence in cases of mild type. He says: "The micrococcus is found in the contents of pustules and vesicles, and also in the blood taken from the measles-papule in mild cases, without its being present in the blood taken from the punctured finger. In severe cases, called malignant in this paper, owing to the rapid appearance of morbid symptoms, the blood shows, early in the attack, numerous patches of micrococcus in the field." These observations were verified by Formad.

PLEURO-PNEUMONIA. — The infectious disease of cattle known as pleuro-pneumonia has been studied experimentally by Willems, Banti, Bouley, Leblanc, Bruylants, Verriest, and others, and strong evidence has been adduced in favor of the view that it is due to a parasitic micro-organism. In 1852 Willems pointed out the existence of certain peculiar corpuscles in the lymph obtained by incision of the lung of an animal dead from this disease. This observation has been confirmed by others, and Bruylants and Verriest describe the organism, which they were able to cultivate in sterilized fluids, as a micrococcus, sometimes isolated, sometimes in pairs, and sometimes in chains of 3–10 elements. The form is slightly oval, and the size varies considerably, the largest measuring 1 μ in diameter.

Protective inoculations are successfully practised in this disease.

INFECTIOUS PNEUMONIA.—That there is an infectious form of pneumonia in man is now pretty generally admitted upon clinical evidence; and several observers have described micro-organisms supposed to bear a causal relation to this disease. Klebs claims to have produced lobular pneumonia in rabbits by injecting the sputum of patients suffering from pneumonia, in which he found an organism called by him *monas pulmonale.* Friedländer, also, found micro-organisms in eight successive cases in the expectoration and in sections of pulmonary tissue. These micrococci were elliptical in shape, one micro-millimetre in length, and two-thirds μ in breadth. They were usually in pairs, but also occurred in chains, and were found most abundantly in the fibrinous expectoration, and in grayish-red hepatization.

The writer would call attention to the fact that these oval micrococci seem to resemble closely those found in the blood of a rabbit killed by the subcutaneous injection of human saliva. (See Fig. 3, Plate

IX., and also p. 359.) Leyden has demonstrated the presence of numerous micrococci corresponding with those described by Friedländer in exudation fluid obtained during life from a patient suffering from severe croupous pneumonia. The fluid was withdrawn by means of a hypodermic syringe. Günther, also, has obtained the same result from an exploratory puncture of hepatized lung. On the other hand, negative results were obtained by Leyden in two milder cases of pneumonia in which fluid was withdrawn from the inflamed lung; and in an epidemic described by Kühn, search for micro-organisms gave a negative result.

PYÆMIA IN RABBITS, *Koch.* — After failing to produce a general infection in rabbits by the injection of putrid blood, Koch succeeded with a fluid obtained by macerating for two days in distilled water a bit of the skin of a mouse. The animal died at the end of one hundred and five hours, and a purulent infiltration of the subcutaneous cellular tissue was found, extending from the point of inoculation as far as the hip behind, and to the middle of the belly below. The peritoneal cavity contained a turbid fluid, and its walls were covered in places by white patches. The liver was covered with a fibrinous exudation, and presented a grayish mottled appearance; upon section it showed gray, wedge-shaped patches. In the lungs were found dark red patches, the size of a pea.

A syringeful of blood from this animal killed a second rabbit in forty hours. Rabbit No. 3 was killed in fifty-four hours by three drops of blood from No. 2; one drop from No. 3 killed No. 4 in ninety-two hours; one-tenth of a drop from No. 4 killed No. 5 in one hundred and twenty-five hours. The pathological appearances in this series of rabbits were similar to those noted in the first, viz.:—

"Local purulent œdematous infiltration of the subcutaneous cellular tissue; metastatic deposits in the lungs and liver; swelling of the spleen and peritonitis. These appearances harmonize so closely with those commonly designated as pyæmia that I do not hesitate to use that term for the disease under consideration.

"On microscopic examination micrococci are found in great numbers everywhere throughout the body, and more especially in parts which have undergone alterations visible to the naked eye. These micrococci are, for the most part, single or in pairs, and their measurement is therefore difficult. Ten measurements of pairs of micrococci differed but little from each other, and gave .25 μ. as the average diameter of a single individual."

It will be noticed that this is much less than the size of the oval micrococcus which produces septicæmia in rabbits.

"As regards size, therefore, they stand midway between the chain-like micrococcus of the progressive gangrene of the tissues and the zooglœa-forming micrococcus of the cheesy abscesses of rabbits. Their relation to the blood-vessels can be best seen in the renal capillaries, and I have therefore selected a small vessel from the cortex of the kidney for delineation (Fig. 18). . . .

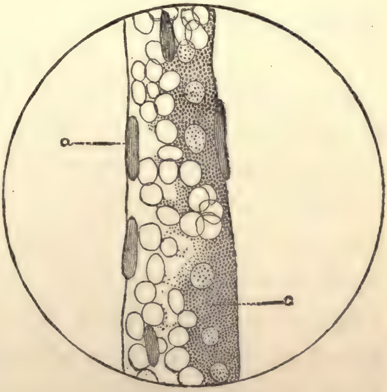

Fig. 18.

"In the interior of the vessel, at *c*, is a dense deposit of micrococci adherent to the wall, and enclosing in its substance a number of red blood-corpuscles. This mass would probably have very soon filled the calibre of the vessel; for fresh corpuscles are constantly being deposited upon it, and these become surrounded by delicate offshoots from the mass of micrococci. From this we may conclude, either that the micrococci have of themselves, owing to the nature of their surface, the power of causing the red blood-corpuscles, to which they adhere, to stick together, or that these organisms can occasion coagulation of the blood in their vicinity, and thus the formation of thrombi. . . .

"Such partial or complete thrombus formations occur in the renal vessels in many places, particularly in the glomeruli, where individual capillary loops may be found completely blocked by micrococci. . . . In the larger vessels, also, groups of considerable size are formed, and I am disposed to believe that the large metastatic deposits in the liver and in the lungs do not arise by gradual growth of a mass of micrococci, but by the arrest of large groups of micrococci and of clots associated with them, formed in the manner described, in the circulating blood; in other words, by true embolism."[1]

Relapsing Fever.—The presence in the blood of patients suffering from relapsing fever of a parasitic micro-organism of spiral form, and exhibiting active movements, was discovered by Obermeier in 1868. Since this date numerous observers have confirmed the discovery, and have verified the fact that this parasite is uniformly found in the blood, in this disease, when the fever is at its acme, both during the first invasion and the relapse. It disappears very quickly, however, when defervescence occurs. These spiral filaments (see Fig. 3, Plate X.) are extremely slender, the diameter never exceeding 1 μ. Their length is from 150 to 200 μ.

"Their motion is very lively, rotatory, twisting, and rapidly progressive; but soon ceases under the ordinary conditions of microscopic examination. As the blood under examination cools and begins to coagulate, these movements become slower, and many spiral filaments become covered with very fine threads of fibrine" (Lebert).

Inoculation of monkeys with the blood of relapsing fever-patients has been successfully practised by Koch and by Carter. Both of these experimenters have also succeeded in cultivating the spirochæte external to the living body.

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[1] Traumatic Infective Diseases, Sydenham Society's translation, p. 51.

As the result of numerous experiments upon monkeys, Carter arrives at the following conclusions:—

> "1. The spirillum fever (relapsing fever) of man is directly transmissible to a quadrumanous animal. 2. There occurs a non-febrile infection of the blood prior to 'fever.' 3. Though the blood-spirillum was never seen in the monkey without fever ensuing sooner or later, yet the pyrexia is secondary in time, and is susceptible of highly varied manifestations; and the spirillum-disease might be defined as essentially a *mycosis sanguinis cum febre*."

Motschutkoffsky has performed inoculation experiments upon man, and was successful with blood taken during the pyrexia; while apyretic blood, milk, urine, etc., gave negative results. According to Heidenreich, "The addition of equal parts of water to the blood is fatal to the spirochæte. Its activity is not affected by any internal administration of quinine, salicylate of soda, or other agents, and externally only affected by about one per cent of quinine."[1]

The evidence in favor of the essential etiological relation of *Spirochæte Obermeieri* to the form of fever with which it is associated is very strong, independently of the confirmatory experimental evidence.

We have here a peculiar parasite invading the blood in very great numbers during the access of the fever; the uniform presence of which, during the first invasion and the relapses, has been verified by numerous observers in various parts of the world. Inasmuch as the blood of healthy persons is free from bacterial organisms of any kind, and as this peculiar organism is not found in any other febrile affection, the presumption is altogether in favor of its causal relation. Looking at it from another point of view, it is difficult to believe that the vital fluid could be invaded by myriads of active parasitic organisms, which must appropriate to their own use material required to preserve the integrity of the circulating fluid and for the nutrition of the tissues, without some disturbance of the economy resulting. In other words, we can easily understand that the presence of the spirochæte might give rise to the fever and other phenomena of the disease; but there is nothing in our experience to indicate that fever causes the appearance in the blood of parasitic organisms of this description.

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[1] Quoted from Shattuck, in Supplement to Ziemssen's Cyclopædia.

The evidence in this case is very different from that relating to the presence of micro-organisms in morbid products of a low grade of vitality found during life, or the demonstration *post mortem* of similar organisms in the blood or tissues. And while we may demand, as final proof, that the disease shall be produced by inoculation with a "pure culture" of the parasite, yet, in the absence of such demonstration, it must be admitted that the evidence is very convincing as to the causal relation of *Spirochœte Obermeieri* to the disease in question.

SCARLET FEVER. — " Coze and Feltz have found in the blood of scarlet fever, taken from patients, living or recently dead, some rods as well as mobile points. This blood injected into the cellular tissue of rabbits has sometimes produced death, and the blood of the animals experimented upon has presented the same bacteria as human blood of scarlatina: they are simply a little larger and longer. As to the mobile points, they appear to correspond to the micrococcus of scarlatina described by Hallier" (Magnin). Reiss found, in blood drawn from a vein in the arm of a patient dying of scarlet fever, that " the serum was filled with an infinite number of small, rapidly oscillating bodies, which, under a magnifying power of five hundred diameters, appeared as black points between the groups of blood corpuscles. In addition, there were also rod-like formations, which at many places were recognized as being composed of three or four or more of these minute bodies disposed in rows." Reiss injected a few drops of this blood under the skin of the back of a rabbit, with the effect of developing like small bodies in its blood, and causing death in twenty-four hours. Further inoculations with this rabbit's blood gave rise to identical results.[1] In the experiments of Coze and Feltz, the introduction of a small quantity of scarlatinous blood beneath the skin of rabbits proved fatal to sixty-two out of sixty-six animals experimented upon. (*Query:* Was this blood obtained *post mortem*, or during the life of the patients?)

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[1] Thomas in Ziemssen's Cyclopædia.

The evidence that the rabbits, in the experiments referred to, suffered a genuine attack of scarlet fever, is not satisfactory; and it must be remembered, in estimating the scientific value of such experiments, that rabbits are very subject to infectious forms of septicæmia; and that the blood of man and animals, obtained *post mortem* from a variety of acute febrile diseases, will produce similar results. On the other hand, it must be admitted that, in its short period of incubation and in other particulars, malignant scarlet fever resembles the infectious forms of septicæmia in the lower animals, shortly to be described; and that septicæmia in man is sometimes attended with a scarlet eruption resembling exactly that which characterizes the disease under consideration.

The occurrence of disease, supposed to be identical with scarlet fever in man, among the domestic animals, — horses, dogs, cats, swine, — has been noted by several observers; and in certain cases communication of the disease by contagion has been traced. "Thus Heim observed that a dog which had lain in the same bed with a scarlatinous child, was taken with fever, followed by scarlatina and desquamation."[1]

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[1] Thomas in Ziemssen's Cyclopædia.

The resources of modern science have not yet been fairly brought to bear for the elucidation of the etiology of this pestilential disease, which in all countries contributes so large a share to the mortality among young children; and it is to be hoped that some government, more liberal in this direction than is that of the United States, may undertake a thorough experimental investigation, in the interests of its citizens, if the advancement of science *per se* is not a sufficient motive. The unsatisfactory results heretofore attained are doubtless to be ascribed to the fact that the difficulties connected with the solution of the problem are too great to be met by individual enterprise, and also to the fact that no amount of enthusiasm can take the place of skill and experience in investigations of this nature. Enough has been done to show that the persistent efforts of trained experts, supported by liberal government patronage, will be required for the settlement of the more difficult problems in etiology.

SEPTICÆMIA IN MICE, *Koch.* — Koch at first failed to produce an infectious disease in mice by the subcutaneous injection of putrid fluids, — blood, meat infusion, etc., — although the injection of a sufficient quantity of these fluids produced death in a few hours. Thus five drops of putrid blood caused the death of a mouse in four to eight hours, and the symptoms of poisoning were developed immediately. But no bacteria were found in blood taken from the heart, or in the internal organs of a mouse killed by such an injection; nor did its blood, taken from the right auricle, cause the death of other mice into which it was injected. The symptoms of poisoning in these cases were more or less severe according to the amount of septic material introduced, and no doubt were due to the chemical poison, sepsin, which is present in putrid blood. But when small quantities of this putrid blood were injected, it happened that, while a majority of the little animals experienced no perceptible effects from the injection, a certain number fell ill at the end of twenty-four hours, and death occurred in forty to sixty hours from the time of inoculation.

In these cases the symptoms and *post mortem* appearances were of a definite character, and the disease was proved to be infectious. This was shown by inoculation from mouse to mouse of a minute quantity of blood, — one-tenth of a drop was ample. Koch says: "I have performed these experiments on fifty-four mice, and have always obtained the same result. Of these, seventeen inoculations were made in succession."[1] We must refer the reader to Koch's work for the symptoms and pathological appearances which characterize this infectious disease; its etiology alone concerns us here.

The certainty with which the infective material can be carried from one animal to another is said to be even greater than in anthrax. In order to infallibly bring about the death of one of these little animals within the time stated,—about fifty hours,—it is sufficient to pass the point of a scalpel, which has been in contact with the infected blood, over a small wound in the skin.

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[1] Traumatic Infective Diseases.

Koch suspected that this great virulence was due to the abundant presence of a micro-organism in the infectious material, but failed in his earlier efforts to find this parasite in septicæmic blood. This was found, later, to be owing to the minute size of the bacilli to which the disease is ascribed; and by the use of Abbe's condenser he was able to demonstrate the presence in large numbers of the bacilli seen in Fig. 19, which is copied from his work (*l. c.*).

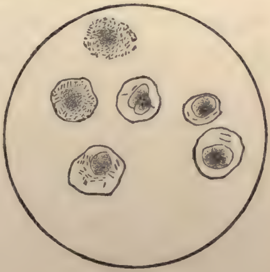

Fig. 19.

White blood-corpuscles from one of the veins of the diaphragm of a septicæmic mouse. × 700.

"The bacilli lie singly or in small groups between the red blood corpuscles, and have a length of .8 to 1 μ. Their thickness, which cannot be measured accurately, but only approximately estimated, is about .1 to .2 μ. . . . One often sees the bacilli in septicæmic blood attached to each other in pairs, either in straight lines or forming an obtuse angle. Chains of three or four bacilli also occur, but they are rare. . . . Without the use of staining materials, the bacilli can only with extreme difficulty be recognized in fresh blood, even when one is familiar with their form; and I have not been able to obtain any certain evidence as to whether they move or not. Their relation to the white blood corpuscles is peculiar. They penetrate these, and multiply in their interior. One often finds that there is hardly a single white corpuscle in the interior of which bacilli cannot be seen. Many corpuscles contain isolated bacilli only; others have thick masses in their interior, the nucleus being still recognizable; while in others the nucleus can be no longer distinguished; and finally the corpuscle may become a cluster of bacilli, breaking up at the margin, — the origin of which one could not have explained had there been no opportunity of seeing all the intermediate steps between the intact white corpuscle and these masses (Fig. 19). Starting from the point of inoculation, one can easily see the path by which the bacilli have penetrated into the body. In the subcutaneous cellular tissue in the neighborhood of the inoculated spot they are very numerous, and at times accumulated in dense masses, as can be best observed in inoculations on the ear. . . . I have never found these bacilli in the lymphatic vessels. . . . I have not found them free in the cavities of the body. . . . In the capillaries the bacilli congregate, particularly at the points of division; but I have never yet seen a complete obstruction of the smaller vessels produced in this way. . . . In exactly the same manner are the bacilli distributed in the rest of the vascular system. In the examination of sections of lung, liver, kidney, and spleen, one meets everywhere with vessels containing free bacilli, and with white blood corpuscles with bacilli in their interior. . . . The whole morbid process has thus a great resemblance to anthrax. In both diseases the infective power of the blood is due to the bacilli present in it; as soon as these disappear, the disease can be no longer produced by inoculation with the blood. Both diseases are distinguished by the invariable development of exceedingly numerous bacilli. There can thus be no doubt that the bacilli of the septicæmia described here possess the same significance as the bacilli of splenic fever, namely, that they are to be regarded as the contagium of this disease."

Very interesting are the results obtained by Koch in his attempts to infect other animals with the blood of septicæmic mice. The rabbit, so susceptible to anthrax and to other forms of septicæmia, resisted not only inoculations with small amounts of the virulent blood, but the entire amount of blood from a septicæmic mouse failed to produce any effect. Field mice, also, although so closely resembling house mice, upon which the successful experiments were made, proved not to be susceptible to the disease.

Septicæmia in Rabbits. — The writer discovered accidentally, in September, 1880, the virulent properties of his own saliva when injected into rabbits, and has since demonstrated the fact that the highly infectious disease which results from such an inoculation is due to a micrococcus constantly present in the buccal secretions, — i. e., in the mixed secretions as found in the mouth. The experiment which led to this discovery was made as a check upon other inoculation experiments, with a view to ascertain whether a fluid supposed to be innocuous would produce any noticeable febrile disturbance when injected beneath the skin of a rabbit. The unexpected death of the animal led to a repetition of the experiment, with the same result, *except when the animal experimented upon had previously been inoculated with various fluids containing bacteria.* These exceptions will be referred to later.

The question at once arose in the writer's mind as to whether the virulence of his saliva, as shown by these experiments, was an individual peculiarity, due perhaps to some antecedent event in his personal history, — e. g., an attack of yellow fever experienced in 1875; or whether it was due to circumstances relating to his environment at the time, — e. g., residence in a Southern city during the summer months, and constant contact with putrefying organic material in the course of his experimental studies; or whether it was, possibly, a general fact that human saliva is fatal to rabbits, when injected beneath their skin.

These questions could evidently only be settled by the experimental method, and a visit was made to the city of Philadelphia, during the month of January, 1881, for the purpose of pursuing the investigation, with the kind assistance of Dr. Formad, in the laboratory of the Medical Department of the University of Pennsylvania. Here, eleven inoculation experiments demonstrated (*a*) that the virulence noted was not due to season or to locality, — as the same result followed inoculations made in Philadelphia during the winter months as had been obtained by similar inoculations in New Orleans during the heat of summer; (*b*) that this virulence was not an individual peculiarity, inasmuch as eleven rabbits, inoculated with the saliva of six different persons, gave eight deaths and three negative results. As no account was made of the previous history of these rabbits, it is now impossible to say whether these negative results are to be ascribed to a less degree of virulence of the saliva injected, or to antecedent experimental injections which had possibly been made in the laboratory, and which afforded these animals protection. Still, a difference in the degree of virulence was shown by the fact that in these, and in numerous subsequent experiments, the writer's saliva has never failed to kill *unprotected* rabbits within forty-eight, or at most sixty hours; while in a considerable number of experiments with the saliva of other persons, there have been several failures to kill; and in other cases the fatal result has been delayed to three or four days, and even longer. This difference could not be accounted for as being connected with unsound teeth or the use of tobacco. The writer has sound teeth, and the secretions which accumulate in his mouth are normal in appearance and reaction, and free from any odor.

The facts thus far observed seemed to be worthy of fuller investigation, with a view to explaining the cause of this virulence; and in the month of March further experiments were commenced in the biological laboratory of Johns Hopkins University. The result of these was very definite, and experimental proof was obtained that the fatal result is due to the presence of a micrococcus in the saliva, which finds the conditions favorable for its rapid multiplication when introduced beneath the skin of a rabbit, and which gives rise to an infectious form of septicæmia, in which, owing to its presence in the blood of an animal recently dead, a minimum quantity of blood taken from the heart of a victim to the disease, is infallibly fatal to other rabbits when introduced in like manner into the subcutaneous cellular tissue. The evidence in support of the etiological *rôle* of the micrococcus in this induced septicæmia of the rabbit is of the same nature as that, just recorded, in the form of septicæmia of the mouse studied by Koch, and as that by which the anthrax bacillus has been shown to be the cause of anthrax. It may be summarized as follows: —

(*a*) The poison is proved to be particulate by filtration experiments.

(*b*) The virulent fluids, saliva, blood, culture-fluids, all contain a micrococcus. (See Figs. 1 and 3, Plate IX.)

(*c*) These fluids produce an identical result, and this result does not vary according to the quantity of material introduced, as is the case where poisonous properties depend upon the presence of a chemical poison.

(*d*) Those agents which destroy the vitality of the micrococcus destroy the virulence of the fluids containing it.

(*e*) *Pure cultures of the micrococcus are as virulent as the saliva, in the first instance, or the blood of a rabbit killed by introducing this fluid beneath its skin.*

I have usually injected from 5 to 20 minims of saliva (mixed salivary secretions and buccal mucus as found in the mouth), and, as stated in my first report, this has has infallibly proved fatal (to unprotected animals). But in an experiment made in Baltimore, a single minim of saliva mixed with five minims of distilled water was injected into each of five young rabbits. Three of the five died within the usual time—forty-eight hours—with the usual symptoms, and presenting the characteristic pathological appearances. The other two showed no ill effect from the injection.

The following quotation from my first report shows the character of this fatal infectious disease, which, originating, as in the above-mentioned experiment, from the introduction of a single drop of human saliva beneath the skin of one of these animals, may be transmitted indefinitely from one to another by successive inoculations.

"The course of the disease and the *post mortem* appearances indicate that it is a form of septicæmia. Immediately after the injection there is a rise of temperature, which in a few hours may reach 2° to 3° C. (3.6° to 5.4° Fahr.); the temperature subsequently falls, and shortly before death is often several degrees below the normal. There is loss of appetite and marked debility after twenty-four hours, and the animal commonly dies during the second night or early in the morning of the second day after the injection. Death results still more quickly when the blood from a rabbit recently dead is injected. Not infrequently convulsions immediately precede death.

"The most marked pathological appearance is a diffuse inflammatory œdema or cellulitis, extending in all directions from the point of injection, but especially to the dependent portions of the body. Occasionally there is a little pus near the puncture, but usually death occurs before the cellulitis reaches the point of producing pus. The subcutaneous connective tissue contains a quantity of bloody serum, which possesses virulent properties, and which contains a multitude of micrococci. There is usually more or less inflammatory adhesion of the integument to the subjacent tissues. The liver is sometimes dark colored and gorged with blood, but more frequently is of a lighter color than normal, and contains much fat. The spleen is either normal in appearance or enlarged and dark colored. Changes in this organ are more marked in those cases which are of the longest duration. In certain cases dark-colored pigment has been found in the spleen, resembling that which has been supposed to be characteristic of malarial fever. The blood is dark-colored, usually fluid, and there is a tendency to agglutination of the red corpuscles.

"The blood commonly contains an immense number of micrococci, usually joined in pairs, and having a diameter of about 0.5 μ. These are found in blood drawn from superficial veins, from arteries, and from the cavities of the heart immediately after death, and in a few cases their presence has been verified during life. Observations thus far made indicate, however, that it is only during the last hours of life that these parasites multiply in the circulating fluid, and in a certain proportion of the cases a careful search has failed to reveal their presence in *post mortem* examinations made immediately upon the death of the animal. This organism, however, is invariably found in great abundance in the serum which exudes in considerable quantities from the œdematous connective tissue when an incision is made through the integument over any point involved in the inflammatory œdema extending from the original puncture."

In this, as in other infectious diseases, the final proof that micro-organisms present in infective material are the cause of the train of morbid phenomena constituting the disease, is to be obtained only from inoculation experiments with pure cultures of these micro-organisms. This proof was obtained for the disease in question during my Baltimore experiments (1881), and a repetition of these experiments in San Francisco (1882) has fully confirmed the results first reported, as is shown by the following record of experiments: —

"*Exp. No.* 1. — San Francisco, July 6, 1882. Injected twenty-five minims of my own saliva beneath the skin of left flank of each of two half-grown rabbits. *Result.* — Both rabbits were found dead on the morning of July 8. *Post mortem* examination at 8 A. M. showed extensive cellulitis, dilatation of superficial veins, and abundant effusion of serum in subcutaneous connective tissue. This serum and the blood obtained from the heart, swarmed with micrococci exactly resembling those heretofore found under similar circumstances in New Orleans, Philadelphia, and Baltimore.[1] One rabbit was still warm, the other had evidently been dead for several hours. The spleen of the first was but slightly enlarged, that of the second was swollen, hard, and dark-colored in patches. No pigment found in either spleen.

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[1] See Special Report to Nat. Board of Health in Bulletin N. B. of H. April 30, 1881.

"A culture-flask containing sterilized rabbit *bouillon* was inoculated with blood from the heart of rabbit No. 1. At the end of twenty-four hours the fluid in this flask swarmed with micrococci. A second culture-flask was inoculated from this, a third from the second, and so on to the sixth, twenty-four hours being allowed in each case for the development of the micrococcus. [The flasks were placed in a culture-oven maintained at a temperature of 100° Fahr. For the author's method of manipulation see p. 177.]

"*Exp. No.* 2. — July 15. Injected twenty-five minims of above culture-fluid (sixth) beneath the skin of a half-grown rabbit. *Result.* — This rabbit died during the night of July 18, and upon *post mortem* examination was found to present the same pathological appearances as in the former experiment, — viz., extensive cellulitis, with effusion of serum swarming with micrococci. The blood also contained the micrococci in abundance; spleen somewhat enlarged and dark-colored; no pigment found.

"A new culture was started from the blood of this rabbit by introducing a minute quantity of blood directly from the left auricle into a culture-flask containing sterilized rabbit *bouillon*. As before, this was carried by successive inoculations from one flask to another to the sixth culture, the culture-flask being in each instance placed in an oven at 100° Fahr., for twenty-four hours, for the development of the micrococcus.

"*Exp. No.* 3. — July 26. Ten minims of above-culture (No. 6) was injected beneath the skin of a half-grown rabbit. *Result.* — The animal died at 10 A. M., July 29, and a *post-mortem* examination was made at once. The subcutaneous cellular tissue was, as usual, infiltrated with serum containing the micrococcus, which was also present in the blood in large numbers. The spleen was very large and dark-colored. A portion was removed for microscopical examination, and the remainder left *in situ*, the animal being so placed that it should be dependent. No pigment was found in the portion first removed, but the presence of black pigment in the portion left *in situ* was verified the following day (removed at 9 A. M.).

"The culture-fluid (No. 6) used in experiment No. 3 (July 26) was laid aside in an hermetically sealed culture-flask until September 12, when a minute drop was used to inoculate sterilized *bouillon* in culture-tube No. 7. This, placed in a culture-oven at 100° Fahr. for twenty-four hours, became clouded, and upon microscopical examination proved to be pervaded by the identical micrococcus heretofore described and photographed. A drop of culture No. 7 was used to inoculate culture No. 8, and the next day, this, being also pervaded by the micrococcus, was used in the following experiment: —

"*Exp. No.* 4. — September 14. Injected ten minims of culture No. 8 into a full-grown rabbit. *Result.* — This animal died at 9 A. M., September 15, and a microscopical examination made at once demonstrated the presence of the micrococcus in great numbers in the blood and in effused serum in the subcutaneous connective tissue. The usual diffuse cellulitis, extending from the point of inoculation, was present; spleen small, and contained no pigment.

"*Remarks.* — This experiment shows that the micrococcus retained its vitality and its full virulence at the end of six weeks; and, very conclusively, that the virulence of the culture-fluid is due to the presence in it of the micrococcus, and not to a hypothetical chemical virus found in the first instance in the saliva, and subsequently in the blood of a rabbit inoculated with this fluid. For the benefit of those who have not calculated the degree of dilution which such a hypothetical chemical virus would undergo in such a series of culture experiments, I submit the following simple calculation: My culture-tubes contain about a fluidrachm of sterilized *bouillon.* The amount of blood introduced into culture No. 1, as seed, was considerably less than a minim, but for convenience I will suppose that one minim is used each time to start a new culture, — that is, the original material is diluted 60 times in the first culture, 3600 times in the second, 216,000 times in the third, and in the eighth culture it will be present in the proportion of one part in 167,961,600,000,000. Yet a few minims of this eighth culture possess all the virulence of the first. . . .

"To convince those who still question the etiological *rôle* of the micrococcus in the infectious disease of rabbits at present under consideration, it would hardly be worth while to carry our culture experiments further, as has been done by Pasteur and other pioneers in this field of investigation, — e. g., in anthrax and in fowl-cholera. I therefore turn to another line of proof.

"I have fixed very definitely the thermal death-point of this septic micrococcus. *It is killed by exposure for ten minutes to a temperature of* 140° *Fahr.* It survives exposure to 130° for the same time. This is the result of a considerable number of experiments, and is established by the simple method of exposing a culture-fluid containing the micrococcus, and enclosed in a hermetically-sealed tube, to a given temperature for the time adopted as a standard, — ten minutes, — and then using the fluid to inoculate sterilized *bouillon* in another tube. This, being placed in a culture oven for twenty-four hours, remains transparent and unchanged if the *seed* has been killed, but is clouded and pervaded by the micrococcus if its vitality was not destroyed.

"In my first series of experiments (Baltimore, 1881) I found that boiling destroys the virulence of blood from a septicæmic rabbit. Having now fixed with precision the thermal death-point of the micrococcus, the next step was evidently to see whether this temperature also destroys the virulence of the fluid containing it. To test this matter, the following experiment was made with the second culture from the blood of the rabbit which died September 15, as above reported.

"*Exp. No.* 5, September 17. — Injected ten minims of culture No. 2 beneath the skin of a small spotted rabbit, also ten minims of the same culture-fluid, heated to 140° Fahr. for ten minutes, beneath the skin of a small white rabbit of the same litter. *Result.* — The small spotted rabbit was found to be dying the following morning at eight o'clock. It was killed by breaking up the medulla, and the blood from the heart examined immediately. This contained the micrococcus in abundance, as did also a quantity of serum contained in the pleural cavity and effused serum in the subcutaneous cellular tissue. The small white rabbit, injected at the same time with the same culture-fluid, *heated to* 140° *for ten minutes*, did not seem to experience the slightest ill effect from the injection, and to-day (September 24) remains in apparent good health; that is, *the virulence of the culture-fluid used in this experiment was destroyed by the exact temperature which I had previously determined to be fatal to the micrococcus.*"[1]

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[1] Quoted from communications to the "Philadelphia Medical Times," of September 9 and November 4, 1882.

If further proof is required, it is to be found in the comparison which the writer has made in his paper on the "Germicide Value of Certain Therapeutic Agents,"[1] of the action of germicides upon the micrococcus as contained in culture-fluids, as compared with the power of the same agents to destroy the virulence of septic blood, as tested by inoculation experiments (*l. c.* p. 342).

It is worthy of remark that, in the very numerous culture-experiments made by the writer at different times and places, in which a sterilized culture-fluid has been inoculated with a minute quantity of blood from the heart of a rabbit just dead from the form of septicæmia under consideration, or from a vein, or from effused serum in the cellular tissue, the micrococcus already described has always been found in the culture after twenty-four hours' incubation, and *it has invariably been found alone*, no other micro-organism having been associated with it in any case. This is offered as very satisfactory proof of the reliability of the method adopted, — i. e., as regards the possibility of accidental contamination; and of the constant presence of this particular micrococcus in the fluids mentioned.

Shortly before the publication of the writer's first report relating to this form of septicæmia in the rabbit, Pasteur announced to the French Academy his discovery of a "new disease" resulting from the injection beneath the skin of a rabbit of buccal mucus, gathered by means of a camels-hair brush from the mouth of a child which died in one of the hospitals of Paris from hydrophobia (December 11, 1880). The material was obtained four hours after death; the brush used to collect it was washed out in water, and the fluid injected into two rabbits. These were found dead December 13. Other rabbits were inoculated with blood from these, and their death with the same symptoms proved that an infectious disease had been produced.

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[1] American Journal of the Medical Sciences, No. CLXX., April, 1883.

There can no longer be any doubt that this disease was identical with that which the writer had previously produced by inoculating rabbits with his own saliva; and, consequently, that the natural inference of Pasteur that this "new disease" was due to the fact that the child from whom the material which produced it was obtained had died of hydrophobia, was an error. Subsequent experiments by Vulpian and others soon made it plain that a mistake had occurred, and nothing more has been heard from Pasteur concerning his new disease. But the results reported are entirely in accord with the deductions of the writer as to the etiological *rôle* of the micrococcus.

Pasteur describes this as follows: —

> "This organism is sometimes so small that it may escape a superficial observation. Its form does not differ from that of many other microscopic beings. It is an extremely short rod a little compressed towards the

middle, resembling a figure 8, and of which the diameter of each half often does not exceed a half a thousandth of a millimeter. Each of these little particles is surrounded at a certain focus with a sort of aureole which corresponds, perhaps, to a material substance."

The possibility that this appearance is due to diffraction is considered, but Pasteur inclines to the opinion that in the case in question it is due to a mucous substance which surrounds the organism. (See Fig. 3, Plate IX.)

At the meeting of the French Association for the Advancement of Science, in 1881, Chauveau, in his address as President of the Association, says: "For a moment we hoped that Pasteur had determined thus [by artificial cultivation] the virus of hydrophobia, *but he tells us himself that he has only cultivated a new septic agent*." Koch's recent attack upon Pasteur, in which he makes much of this mistake, seems a little out of place in view of this frank confession made more than two years ago.

The last-named observer has also encountered this form of induced septicæmia in the rabbit, and has shown that the micrococcus which produces it is not alone found in human saliva. This was *a priori* to have been expected, and the writer has never supposed that the human mouth was the only habitat of the micro-organism in question. But being unwilling to generalize from insufficient data, he has not even claimed that *all* human saliva is fatal to rabbits, but has taken pains to say, in recording his results, "*my* saliva" injected in such or such an amount produces, etc.

Koch gives the following interesting account of the occurrence of this interesting disease in the course of his experimental inoculations:—

"After injection of putrid infusion of meat into rabbits, I have twice obtained a general infection of another sort in which metastatic deposits do not occur [as is the case in the disease described by him as pyæmia in rabbits], and which I would therefore describe, in contrast to the foregoing, as septicæmia. This infusion, like the putrid fluids used in the earlier experiments, contained numbers of bacteria of the most various forms. When injected under the skin of the back of a rabbit it produces an extensive putrid suppuration of the subcutaneous cellular tissue, and the animal dies in three days and a half. At the ichorous spot, which must, on account of its size, be looked upon as the immediate cause of death (owing to absorption of poisonous material in solution), the same variety of bacteric forms was present as in meat infusion. At the border of this spot the cellular tissue was infiltrated with a slightly turbid watery fluid which contrasted strikingly with the brownish ichor in the vicinity of the place of injection. In this œdema fluid great numbers of micrococci of considerable size and of an oval form were almost the only organism observed. In the blood also similar micrococci were found, though only in small numbers. Further, in the papilli of the kidney and in the greatly enlarged spleen, some of the small veins were blocked for short distances with these oval micrococci.

"Two drops of this œdematous fluid were now injected under the skin of the back of a second rabbit. The animal died in twenty-four hours, and here, in the neighborhood of the place of injection, not a trace of pus could be observed. On the other hand, slight œdema, with a streaky whitish appearance of the subcutaneous cellular tissue, extended from the point of injection to the abdomen. In this œdematous cellular tissue lay numerous flat extravasations of blood half a centimeter in breadth, the vessels around being greatly distended. The muscles of the thigh and of the abdominal walls were also interspersed with small extravasations. [These hemorrhagic extravasations were common also in the victims of the writer's experiments.] In the heart and lungs no alterations were found. In the peritoneal cavity no fluid was present, the peritoneum being unaltered and the coils of intestine not glued together. But the surface of the intestine, in consequence of a number of small subserous extravasations, presented an appearance as if injected here and there with blood. The spleen was also very considerably enlarged. In this second animal the oval micrococci were alone present in the œdematous cellular tissue, all the other bacteria having disappeared. The number of these organisms was very considerable, many of the small veins being completely filled with them. . . .

"These micrococci differ from the micrococci of pyæmia very markedly as regards size, and in most other points. Thus they never enclose the blood corpuscles, even when they have accumulated in large numbers in the interior of the blood-vessels. They rather push them on one side. They do not cause coagulation of the blood, and thus emboli do not occur."

The experiments made by the writer have been repeated by Claxton, who says: —

"I shall now discuss briefly the second part of my argument, namely, what constituent of the saliva produces the fatal disease? And as my results accord so perfectly with those obtained by Sternberg, and my experiments in this direction are but repetitions of his, I shall be pardoned, I trust, for answering the question in his own words.

"'*The following facts demonstrate that the phenomena detailed result from the presence of a living organism found in the saliva, namely, a micrococcus which multiplies in the subcutaneous connective tissue, and also in the blood shortly before or after death.*'"

This extended account of the disease under consideration, and of the evidence in support of the writer's first announcement as regards its etiology, has been given because rabbits are extensively employed in experiments relating to the etiology of infectious diseases, and it is important that those who enter upon such investigations should be familiar with all forms of disease to which they are subject. And also, because, notwithstanding the experimental evidence adduced in favor of the view that the virulence of normal human saliva is due to the micrococcus described, it has been evident that there has been considerable incredulity as to the correctness of this conclusion, on the part of many worthy members of the profession.

We have seen, in the article on septicæmia in mice, that rabbits are not susceptible to this form of septicæmia, which Koch has shown to be due to a bacillus. On the other hand, Koch found that the injection of blood from a septicæmic rabbit into a mouse, although it killed the little animal in thirty-seven hours, did not give rise to the infectious form of the disease; for a second mouse, which was inoculated with blood from the heart of the first, was not visibly affected.

In a limited number of experiments by the writer, in which his own saliva was injected into animals other than the rabbit, the following results were obtained: —

Injection of 4 c. c. into each of two small dogs produced local abscesses at the point of injection, but no other noticeable results. A dog succumbed, however, to an injection of 1 c. c. of serum from the cellular tissue of a rabbit recently dead.

Injection of 0.25 c. c. (each) into five chickens produced no result.

Injection of 0.75 c. c. (each) into three guinea-pigs proved fatal to two, — one in three, and one in seven days.

Injection of 0.5 c. c. into five rats resulted fatally to one only.

These results correspond with those reported by Pasteur, who found the guinea-pig less susceptible than the rabbit; the chicken entirely insusceptible; and the dog susceptible to injections of blood from dead rabbits.

The value of protective inoculations in this form of septicæmia has been brought out accidentally in the course of the writer's experiments; and it has been his intention to investigate this interesting subject fully by the experimental method. This he has not yet been able to do, and, consequently, can only present such facts as have been developed by experiments made with a different object.

Two rabbits injected with full doses of my saliva, in New Orleans, proved to be insusceptible to its lethal effects. These rabbits had previously received the following experimental inoculations: —

Rabbit No. 1. — Received October 7, 1.35 c. c. of swamp-culture (organisms from swamp mud cultivated in gelatine solution *a la* Klebs and Tommasi-Crudeli); October 28, 1.3 c. c. of spleen-culture (from a rabbit which died from an injection of 0.75 c. c. of swamp-culture in gelatine solution).

Rabbit No. 2. — Received October 7, 1.35 c. c. of spleen-culture; and October 27, 1.26 c. c. of spleen-culture, which injection was repeated the following day.

On the 12th of November these rabbits both received subcutaneously 1.26 c. c. of my saliva, and, except for a slight febrile reaction, experienced no ill effect from the dose.

Baltimore, May 24, 1881, injected into a large rabbit 1.25 c. c. of virus, not disinfected, from a rabbit recently dead. *Result negative.* This rabbit had previously (May 13) received an injection of 0.5 c. c. of virus mixed half an hour previously with sodium hyposulphite in the proportion of one per cent. The virus used in these experiments was bloody serum from a rabbit just dead, which was proved by other experiments to be fatal to unprotected rabbits in the smallest quantity. Thus, the needle of a hypodermic syringe (Exp. of June 2, 1881) was dipped into the blood of a septicæmic rabbit just dead, which was proved by microscopical examination to contain the micrococcus in abundance. This needle was then introduced under the skin of another rabbit, which died within forty-eight hours, and presented the usual appearances of septicæmia.

Protection was also afforded in one case by an injection of virus which had been mixed half an hour previously with three parts of 95 per cent. alcohol.

Finally, I take the liberty of quoting the case of Dr. Formad's famous buck rabbit: —

> "There remained in the laboratory a number of living animals, left over after the various experimenters of my pathological class ceased work, at the conclusion of last winter's term. Among the number was a buck rabbit, which had been largely dosed, by my friend Claxton, with saliva of some kind. Since then, during the last six months, this same rabbit was injected subcutaneously, at different times, with all the articles of the following bill of fare:
>
> "1. Human saliva (second time); 2. Cancer juice; 3. Epidemic diphtheritic material from Michigan; 4. Bouillon, containing a rich crop of cultured micrococci from the same material; 5. Diphtheritic material from a fatal case in the city; 6. Slough from rabbit, dead from diphtheria; 7. Slough from scarlatinal sore throat; 8. Slough from erysipelas; 9. Slough from gangrene; 10. Cadaveric poison; 11. Feces from typhoid fever case; 12. Sputa from case of tuberculosis."[1]

It is pretty evident that this rabbit was protected from septic poisoning; and the case is exceedingly instructive, not only as illustrating the value of protective inoculations against septicæmia, but as showing the importance of selecting rabbits not previously experimented upon for experimental studies relating to the etiology of infectious diseases.

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[1] Philadelphia Med. Times, Sept. 16, 1882, p. 194.

It should also be remembered by those who undertake experimental investigations of this nature, that accidental inoculation may occur, or that a rabbit may suffer a non-fatal attack as the result of contact with other septicæmic animals, or from being placed in infected cages. Davaine long since recorded the fact that spontaneous septicæmia occurred among his rabbits from this cause; and the writer has also lost a number of rabbits in this way, while others of the same lot recovered after a brief illness, and subsequently proved to be protected from the lethal effects of septic virus.

It is not impossible that, in man, a certain immunity from infectious diseases, the epidemic prevalence of which depends upon the presence of decomposing organic material in the infected localities, — e. g., cholera, yellow fever, diphtheria, — may be acquired by exposure to septic material which lacks the infectious character; i. e., that a tolerance is established to the effects of the chemical poison or poisons which are evolved as a result of the vital activity of both pathogenic and non-pathogenic bacteria. It has frequently been noted that grave-diggers, those who clean sewers, and those who pursue pathological studies, are even less liable to contract the diseases mentioned than those members of the community who are not so much exposed to infection.

SPREADING ABSCESS IN RABBITS, Koch: —

"Coze and Feltz, Davaine, and many others, have obtained in rabbits, by the injection of putrid blood, an infective septicæmic disease. I have therefore repeated their experiments. I have not, however, succeeded in producing the effects produced by Davaine, but I observed — what others who have made similar experiments on rabbits have already noticed — that in these animals the formation of an abscess constantly increasing in extent, may occur in the subcutaneous cellular tissue without any general infection taking place. Such animals have at first no symptoms of disease; a flat lentiform hard infiltration at the seat of injection is all that can be observed. After several days this hardness extends in all directions, chiefly downwards, especially towards the abdomen and anterior extremities. The animal at the same time emaciates and grows feeble, and dies in about twelve to fifteen days after the injection.

"The *post mortem* examination shows the presence, in the subcutaneous tissue, of extensive flat abscesses with cheesy contents; their walls bulge in various directions, though the whole remains a single cavity. There is also an extreme degree of emaciation, but no alteration in the peritoneum, intestine, kidney, spleen, liver, heart, or lungs. In the blood the white corpuscles are greatly increased in number, but no bacteria can be found. The cheesy contents consist of a finely granular material, and scattered about in this are nuclei undergoing disintegration; but no bacteria can be definitely made out. Here, then, we have appearances similar to those often found in man, and much used as an argument against the parasitic nature of such morbid processes. I refer to abscesses resulting from phlegmonous inflammation, which must be regarded as infective in their origin, but in which no micro-organisms have been found.

"When, however, portions of these abscesses are hardened, and examined in sections, the surprising result is obtained that, though bacteria are not present in their contents, their walls are everywhere formed by a thin layer of micrococci, united together into thick zoglœa masses. These organisms are the smallest pathogenic micrococci which I have as yet observed. In some places I was fortunate enough to find them arranged in rows, and thus I was able to measure them; and I ascertained that they were about .15 μ in diameter. (This is, of course, only an approximate measurement.) . . .

"In order to ascertain whether the morbid process here designated as progressive abscess formation could be transmitted from one animal to another, rabbits were injected with blood taken from others which had already died of this disease. These injections produced no effect. A small quantity of the cheesy contents of the abscess was now taken, diluted with distilled water, and injected under the skin of a rabbit. These resulted exactly the same, — abscess formation in this animal as in the first. The abscesses spread in the same manner as described in the former case, and caused the death of the animal experimented on in a week and a half. From this animal the disease was conveyed to a third, and so on through several in succession.

"It was thus demonstrated that the disease is not merely occasioned by the injection of a considerable quantity of putrefying blood, but is of a decidedly infective character. The assumption made above, that the micrococci in the cheesy contents of these abscesses are dead, does not appear in keeping with this result of inoculation. This apparent contradiction may, however, I think, be cleared up; for it is very probable that these micrococci, like other bacteria, form resting spores (Dauersporen) after the expiration of their vegetative life, and that these bodies, just like the spores of bacillus, are not stained by aniline, and therefore remain invisible in Canada balsam. The infection in the case referred to would be brought about by such spores."[1]

SWINE PLAGUE; *le rouget ou mal rouge des porcs* (Pasteur); infectious pneumo-enteritis of the pig (Klein). In a recent communication (December 4, 1882) to the French Academy, Pasteur gives the following summary of results obtained in an experimental research relating to the above-mentioned disease: —

"I. Swine plague (*mal rouge des porcs*) is produced by a special microbe, which is easily cultivated outside of the body of the animal. It is so minute that it may easily escape observation, even the most attentive. It most nearly resembles the microbe of fowl-cholera, its form being that of the figure 8. But it is smaller and less easily seen, and differs essentially from the microbe of fowl-cholera in its physiological properties. It has no action upon fowls, but kills rabbits and sheep.

"II. When inoculated in a pure condition into pigs, in quantities almost inappreciable, it promptly gives rise to the disease and to death, the symptoms being the same as in spontaneous cases. It is especially fatal to the white race (improved breed, most highly valued by those who raise pigs).

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[1] **Traumatic Infective Diseases, pp. 45–47.**

"III. In 1878 Dr. Klein, of London, published an elaborate research upon this disease, which he calls infectious pneumo-enteritis of the pig; but this author has been entirely mistaken as to the nature and properties of the parasite. He has described a bacillus with spores as the microbe of this disease, which he describes as being even larger than *Bacillus anthracis* (*la bactéride du charbon*). This is very different from the true microbe of swine plague, and has no relation to the etiology of the disease.

"IV. After assuring ourselves, by direct proof, that the disease does not recur, we have succeeded in inoculating it in a mild form, and the animal has subsequently proved to be protected against the malignant form of the disease."

Néguin and Salmon had previously reported their failure to find the bacillus of Klein in the blood and other infectious fluids obtained from animals sick with this disease, and the constant presence of a minute micrococcus apparently identical with that described by Pasteur.

Salmon says that blood drawn from the veins of a pig affected with swine-plague into "capillary vacuum tubes" was quite free from bacilli at the end of ten days. But this blood swarmed with micrococci, single, in pairs (Pasteur's Fig. 8), in chains, and in zooglœa masses. Healthy pigs inoculated with this blood sickened at the end of seven days and exhibited the characteristic symptoms of the disease. These inoculations did not, however, produce a fatal form of the malady, and Salmon found it impossible to carry the virus beyond a second generation, even by inoculating pigs which had never before been exposed to the contagium. Inoculations with cultivated virus, containing the micrococcus in abundance, produced a discoloration of the skin, and a slight eruption; but the symptoms were not sufficiently definite to enable the experimenter to say with certainty that the inoculated animals suffered a mild attack of the disease.

SYPHILIS. — The presence of bacteria in the initial lesion of syphilis, in secondary papules, in syphilitic new growths, and in the secretions of chancroids and syphilitic ulcers, has been noted by numerous observers. But the descriptions given by different individuals are not entirely in accord as to the morphological characters of these bacteria. According to some, — Hallier, Klebs, Bermann, — they are micrococci; while others have found bacilli, — Birch-Hirschfeld, Morison; and Salisbury finds a fungus — his *Crypta syphilitica* — in the blood as well as in the local lesions of syphilis.

Birch-Hirschfeld at first described the organisms found by him in syphilitic growths as bacilli, but has since become convinced that they are oval micrococci arranged in chains. He says that it is more difficult to distinguish the individual elements in the chains than in the case of spherical micrococci. These oval elements are found single, in pairs, or in chains of four or five, which greatly resemble long rods with rounded ends. This description agrees with that of Aufrecht:

> "For the demonstration of the organisms in recent preparations, Birch-Hirschfeld prefers potash, by the clearing action of which the micrococci are visible in the tissue, on account of their strong refracting power. In a broad condyloma, they lie, for the most part, in small aggregations in the papillæ, and in many of the cells of the adjacent layer of the rete Malpighii. They may be readily detected in the juice of a recently excised condyloma, by tinting in the ordinary way; and of the various staining agents, Birch-Hirschfeld concludes that fuchsin and gentian-violet are the best. In the growths in internal organs the smallest micrococci are most abundant, and the larger forms seen in the condylomata are seldom met with. In gummatous scars they are sought for in vain. In more recent gummatous products they were most abundant in parts which had the aspect of growing granulation tissue. They were partly scattered, partly aggregated into groups, which never exceeded a granulation-cell in size; they were also distinctly seen within the cells. Many epithelioid cells seemed to have their nuclei filled with these organisms."[1]

Dr. Bermann of Baltimore finds in absolutely fresh specimens of indurated chancres, "collections of micrococci and fungoid growths, firmly adhering to and partly blocking the lumina of most of the lymphatic vessels." According to this observer, the micrococci of syphilis are small, strongly refracting bodies; resembling those described by Klebs.

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[1] London Lancet, December 2, 1882.

Recently Dr. Morison of Baltimore has made a careful study of the bacteria found in chancroids and in syphilitic lesions, in the wards of Professor Neumann of Vienna. As he resorted to the most approved methods of staining, and seems to have exercised special care in collecting and mounting his material for microscopic examination, his observations are of value, and I have taken the liberty of copying his figures from the "Maryland Medical Journal" of January 1, 1883, in which his paper was published. (See Figs. 20 and 21.)

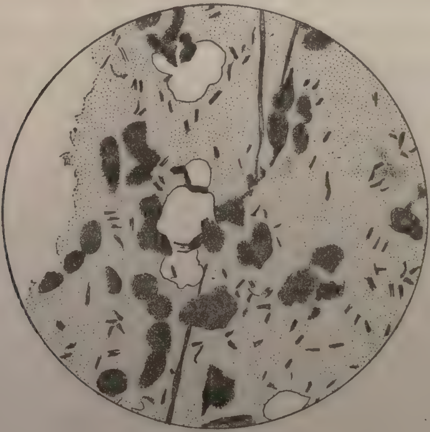

Fig. 20.

Hard chancre secretion with bacteria, magnified 850 diameters. (Drawn by Heitzmann.)

No satisfactory proof has yet been offered in support of the view that any one of the organisms above described is the veritable germ of syphilis;

and it is evident that the greatest caution must be exercised in drawing any conclusions as to their etiological import. For there is nothing improbable in the supposition that tissues of a low grade of vitality may be invaded by parasites which have no causal relation to the morbid process; and in view of what we know of the extended distribution and infinite variety of organisms of this class, their absence from the secretions of an open ulcer would be more remarkable than their presence. In a second communication, dated March 23, Dr. Morison states that a modification of his method of staining has enabled him to demonstrate that the rods seen in Fig. 20 are really formed of closely united cocci, corresponding with those described by Birch-Hirschfeld. He further says: —

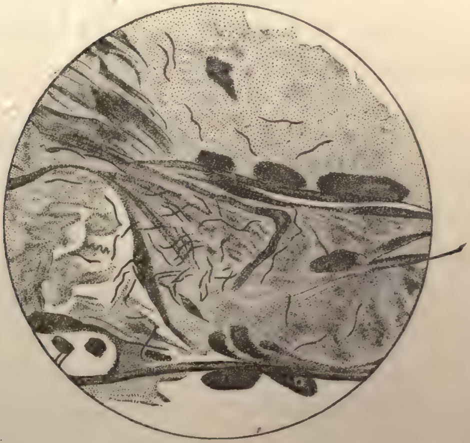

Fig. 21.

Soft chancre secretion with bacteria, magnified 850 diameters. (Drawn by Heitzmann.)

"The result of these recent experiments is such that I am not only forced to deny the pathogenic nature of the micro-organisms described in my first communication, but also to add that I am convinced their presence in the secretions was due to external influences."

Klebs claims to have produced syphilis in the monkey, and Martineau and Hamoine to have communicated the disease to young pigs (Morison). But with these exceptions, so far as the writer is aware, attempts to inoculate syphilis in the lower animals have given negative results.

TUBERCULOSIS.—The experimental researches of Villeman, Tappeiner, Cohnheim, Toussaint, and others, having apparently established the fact that tuberculosis is an infectious disease, the medical profession was not unprepared for the discovery, first announced by Koch in the spring of 1882, of a parasitic micro-organism in tuberculous material, bearing a causal relation to the disease in question. Coming from Koch this announcement had great weight and at once received the most attentive consideration in all parts of the civilized world; for he was already well known to be both a skilful and a cautious investigator.

The experimental proof offered in favor of the view that the bacillus discovered in the sputum of tuberculous patients, and in recent tubercles in the lungs and elsewhere, was the veritable cause of tuberculosis, seemed so convincing, that it might have been received almost without question, but for the fact that other experimenters had previously found that tuberculosis in animals may result from inoculation with a variety of organic products of non-tubercular origin, and even from the inhalation of inorganic particles; which also is recognized as a cause of pulmonary consumption in man. As an example of the numerous experiments of this kind, we may refer to the results obtained by Brunet, who inoculated seven rabbits with cancer, six with simple pus, and six with tuberculous material. Of those, fourteen became tuberculous, namely, six of those inoculated with cancer, three of those inoculated with pus, and five of those inoculated with tuberculous matter.

Schottelius found that miliary nodules in the lungs resulted, in dogs, alike from inhalation of pulverized — spray — sputum of bronchitis and of phthisis.

Toussaint affirms that the tubercular deposits resulting from inoculation with non-tubercular material are not infectious, and that experimental pseudo-tuberculosis may be distinguished from tuberculosis proper by inoculation experiments, although the pathological anatomy of the two diseases is identical.

Koch, on the other hand, does not admit that tuberculosis can be produced by material from which living tubercle bacilli or their spores are unquestionably excluded. In his own experiments he found that in all cases where the material used for inoculation contained living bacilli or spores, the result was positive in animals liable to infection; while when the material inoculated did not contain these bacilli or their spores, a negative result was obtained. Thus, in several cases, experiments were performed with the contents of a scrofulous gland and with various other material proved by examination to be free from bacilli, and in no instance did tuberculosis follow. The positive results obtained by other experimenters with non-tuberculous material are explained by the supposition that tubercle bacilli or their spores have been introduced at the same time. It is evident that this accidental inoculation would be very apt to occur in laboratories where tuberculous animals had been kept under observation, and especially where proper precautions are not taken as regards cleanliness of the cages in which animals are kept, and the isolation of those which are subjected to inoculation experiments.

According to Koch, the tubercle bacillus is a slender rod from a quarter to a half of the diameter of a blood-corpuscle in length, and presents certain distinctive characters as regards its behavior with staining reagents. The various methods of staining this bacillus are given in PART THIRD of the present volume. The bacilli are found in considerable numbers in tubercles of recent formation, more especially at the border of the cheesy masses. They are abundant in the giant-cells, and seem to possess a special relation to these cells. They are not so abundant in old tubercles, although they are seldom entirely absent. By placing a small portion of a recent tubercle in blood-serum or distilled water, they may be recognized with a suitable objective and illuminating apparatus, without the use of staining reagents. An examination made under these circumstances shows that the bacilli are motionless, and in some rods spores of oval form may be distinguished. At the time of his first report, Koch had examined in man, "Eleven cases of miliary tuberculosis, twelve cases of cheesy broncho-pueumonia, one case of tubercle in the brain, and two cases of intestinal tuberculosis." In all of these the bacilli were present. They were also found in freshly extirpated scrofulous glands. Among the lower animals they were found in ten cases of *perlsucht*, in three cases of so-called bronchiectasis in cattle; in three monkeys, nine guinea-pigs, and seven rabbits, which had spontaneous tuberculosis; and in one hundred and seventy-two guinea-pigs, thirty-two rabbits, and five cats, which had been inoculated with tuberculous material, or with pure cultures of the bacillus.

The gelatine culture-medium which had been previously recommended by Koch was found not to be suitable for the cultivation of the tubercle bacillus, as the advantage of solidity is lost when this is heated to 98° Fahr. Jellified blood-serum, prepared as directed on page 163, was found, however, to fulfil all the required conditions, and was used by Koch in his culture experiments. Portions of tubercles removed, with proper precautions to prevent contamination, from the bodies of persons recently dead of tuberculosis, or from the lower animals, victims of spontaneous or of induced tuberculosis, were placed upon the surface of the sterilized blood-serum, and the vessel containing it was kept in a culture-oven maintained at a temperature of 40° C. (104° Fahr.). During the first week no marked alteration occurred, unless other bacteria had gained access to the culture-medium, in which case the experiment was a failure. About the tenth day small points and scales became evident, which slowly spread, and upon microscopical examination proved to consist of tubercle-bacilli. After fourteen days these bacilli were used to start a new culture. This was accomplished by breaking up the scales and transferring a minute quantity to the surface of culture No. 2. After transferring the bacilli in this way to several successive flasks, it was assumed that the original material was excluded, and that a pure culture had been obtained. Inoculation of guinea-pigs with these pure cultures gave rise to tuberculosis with as great certainty as in those experiments in which tubercular material was used. In one experiment six newly bought guinea-pigs were obtained. Two of these were kept as *témoins*, and the other four were inoculated with cultivated bacilli obtained in the first instance from the lung of a human being who had died of miliary tuberculosis. In this instance five successive cultures had been carried out, the time required being fifty-four days. One of the inoculated guinea-pigs died on the thirty-second day, and all the rest were killed on the thirty-fifth day. All had extensive tuberculosis, and the *Bacillus tuberculosis* was found in the tubercles of the lungs, and of various organs. The two guinea-pigs not inoculated remained healthy.

In another experiment four rabbits were taken. Into the eye of one pure blood-serum was injected; the point of a syringe containing tubercle bacilli in blood-serum was introduced into the eye of a second. These were from a series of cultures carried out for 132 days. In this case the piston was not moved; but the same material was injected into the eye of rabbit No. 3, and of rabbit No. 4. The animals were killed on the thirtieth day, and the following result noted: Rabbit No. 1 remained healthy; rabbit No. 2 had typical tuberculosis of the iris, and the nearest lymphatic glands were swollen and infiltrated with yellowish nodules; but the lungs and other organs were free from tubercles. Rabbits Nos. 3 and 4 had iritis and tuberculosis of the lungs.

The presence of Koch's bacilli in tuberculous sputum has now been confirmed by numerous observers in various parts of the world; and the comparatively few failures to find the bacillus which have been reported by expert manipulators since the method of Ehrlich was published, are easily accounted for in other ways than upon the supposition that cases of tuberculosis occur in which no bacilli are found. Nevertheless we must admit that there are cases, recognized by expert pathologists as undoubtedly tubercular, in which no bacilli can be found in tubercles obtained from the lungs *post mortem*. Thus Prudden, of New York, while recording the fact that he has, in a considerable number of cases of acute and chronic phthisis, found, almost invariably, the bacillus of Koch "in and about all of the cavities, in many of the larger areas of coagulative necrosis, and in a considerable proportion of the miliary tubercles;" yet reports two cases which form an exception to this rule. In one, an abundance of miliary tubercles covered the lateral surfaces of both lobes of the left lung; "most of these were of the usual giant-celled and epithelioid-celled type, with a more or less well-marked reticulum. In none of those examined was there well-marked cheesy degeneration. Six hundred and ninety-five sections, about .01 millimeter in thickness, were made from ninety-nine different tubercles from various parts of the tuberculous membrane, and stained in the usual manner by Ehrlich's method in several different lots. In not one of these six hundred and ninety-five sections could a single tubercle bacillus be detected, although all were examined with the most scrupulous care." In another case, "nine hundred and nine sections from a large number of peritoneal tubercles, from different parts of the affected surfaces, stained by Ehrlich's method, revealed, under the most searching scrutiny, no tubercle bacilli." In the same case, however, nodules at the apex of the lung, and the wall of a small cavity formed of shreds of necrotic tissue, of dense cheesy material, and in the outermost layers of tubercle tissue and ordinary dense connective tissue, proved to contain the bacillus in abundance *in the walls and edges of the cavity*, and in a few of the dense areas of coagulation necrosis in its immediate vicinity. But in the diffuse tubercle tissue, in the zones of simple pneumonia around the nodules, in the scattered fibrous tubercles in the lung and pleura, and in the well-formed tubercles in the bronchial glands, no bacilli could be found.

Koch has received ample confirmation as to the presence of the bacillus described by him, in phthisical sputum; and its absence from the sputum of patients suffering from other diseases seems to be pretty well established, although Spina of Vienna claims that other bacteria behave precisely towards staining agents as do the bacilli of Koch; and, consequently, that the color-test cannot be relied upon for distinguishing this bacillus from the ordinary bacteria of putrefaction. The writer's observations are entirely in favor of the statement of the discoverer of the tubercle bacilli as to their peculiar color reaction when treated by Ehrlich's method; but, like many others, he has not been successful in demonstrating them by the method first proposed by Koch.

A recent writer[1] has collected the statistics, as published in various journals, and states that in 2,509 cases reported, the bacilli were found in 2,417. Koch, himself, recognizes, however, that this kind of evidence cannot be taken as proof of the causal relation of the bacillus to the morbid process which results in the formation of tubercles in various parts of the body. For it may be that the bacillus is present in tuberculous material simply because this furnishes the pabulum necessary for its development, and is absent from the sputum of bronchitis, for example, because this does not constitute a suitable culture-medium; or because, being secreted from the surface of an inflamed mucous membrane, and quickly removed by expectoration, there is no time for the development of this bacillus, which Koch has shown requires at least a week before any evidence of multiplication is seen upon the surface of sterilized blood-serum. This time would, however, be afforded in the cheesy contents of a tubercular nodule, or in a cavity where necrotic products were retained for a considerable time.

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[1] Dr. Ferguson, of Canada. See Med. Record, New York, July 21, 1883, p. 77.

A recent French writer, Cochez, claims that the sputum of phthisical patients constitutes a favorable culture-medium for the tubercle bacillus. The writer, also, has been inclined to believe that the bacilli are more numerous in sputum which has been kept for a day or two than in the same material when first obtained. This, if true, is not very favorable to the view that they are the cause of the morbid process which results in the formation of miliary tubercles, although by no means directly opposed to this belief.

An interesting communication relating to the finding of Koch's bacillus in pathological specimens which have undergone putrefaction, or in those which have been kept for some time in preservative solutions, has recently been made by Vignal. This author finds that "putrefaction, even very much advanced, does not seem to interfere with finding the tubercle bacillus. They are also found as easily in pieces kept a long time in 90 per cent and absolute alcohol, and in Müller's fluid, as in recent preparations."

The morphological characters of the tubercle bacillus, as found in sputum, are delineated in Fig. 22. The bacilli are found both within and without the pus-cells, and seem to be especially numerous in the epithelioid cells. They vary greatly in length, and are not infrequently curved or bent at an angle more or less acute. Not infrequently they occur in pairs, or in little groups, and in some cases it is apparent that they contain endogenous spores, or that they are made up of a chain of oval elements. In my "Photo-Micrographs,"

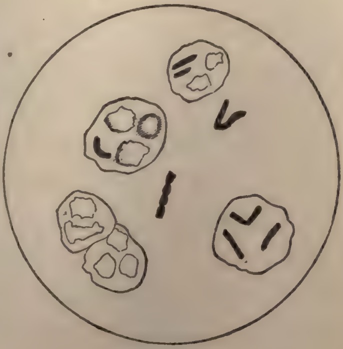

Fig. 22.

Koch's *Bacillus tuberculosis*, in sputum; stained by Ehrlich's method. × 1000 (G. M. S., del.).

Figs. 3 and 6, Plate XI., the bacillus is seen very indistinctly; but a close inspection of these figures will show a single bacillus containing spores in Fig. 6; and in Fig. 3 groups of two contained in an epithelioid cell, the outlines of which can barely be distinguished. Under the microscope these bacilli, which had been stained with fuchsin by Ehrlich's method, were beautifully shown as bright-colored rods, which contrasted strongly in appearance with the decolorized micrococci and putrefactive bacteria in the same specimen. But after a considerable number of trials, no better photographic result could be obtained than that here seen.

The fact that the tubercle bacillus has not invariably been found by competent observers in recent tubercles, and, also, that numerous investigators claim to have produced tuberculosis in susceptible animals by inoculating them with non-tubercular material, must make us unusually exacting as regards the experimental evidence furnished by inoculations with pure cultures. The writer has already indicated his reasons for thinking that a series of cultures made upon the *surface* of a solid culture-medium is less satisfactory, as regards the exclusion of the original material, than where a fluid culture-medium is employed, which is pervaded throughout by the organism under cultivation. (See p. 311.)

The force of this criticism will be more fully appreciated when it is remembered that Koch uses several bits of a recent tubercle to start a culture upon the surface of jellied blood-serum. It certainly seems that, under these circumstances, four or five successive cultures are not sufficient to make it entirely certain that we have obtained a pure culture of the bacillus; and we may fairly demand that the culture experiment should be carried much beyond this point. This requires, however, an amount of skilful manipulation, and of patient waiting which it will be hard to obtain outside of Koch's laboratory; and especially in this country, where no government aid is extended to those who engage in investigations of this character. When stimulated by the expectation of making a new discovery which will make his name famous, a man may accomplish wonders in exploring difficult and unknown territory. But when the ground is to be re-surveyed, and carefully mapped, it is not to be expected that individual enterprise will be equal to the undertaking. The writer speaks with feeling in this matter, for he realizes how imperfect his own attempt to repeat Koch's experiments has been.

A summary statement of the results obtained in this attempt is given below, as originally reported in "The Medical News" of December 30, 1882: —

> "If I have not been able to answer the question, *Is tuberculosis a parasitic disease?* the experimental data thus far furnished will at least be of use to the jury — the medical profession — in making up a final verdict; which, I submit, would in the present stage of the in-

vestigation be premature. I therefore beg leave to retire from the witness-stand, and to take my seat with the jury, with the understanding that I may be recalled if circumstances enable me to add to the testimony already given. First, however, it may be well to make a summary statement of the evidence presented; which I do as follows: —

"(*a*) The bacillus described by Koch is found in the sputum of phthisical patients in San Francisco.

"(*b*) That this bacillus differs from the micro-organisms found in normal human saliva and in bronchitic sputum, is proved by the color-test (Ehrlich's) and by culture-experiments (see below, *i* and *j*).

"(*c*) Tuberculosis in animals may result from inoculation — subcutaneous — with the sputum of tuberculous patients.

"(*d*) But in several of the animals experimented upon, no evidence of tubercular deposit was found, and in others it was very slight. Total number of animals experimented upon, twelve rabbits, two guinea-pigs, and two dogs.

"(*e*) In the animals successfully inoculated, the enlarged tuberculous lymphatic glands in the vicinity of the point of inoculation, and tubercular nodules in the lungs and elsewhere, *usually* contained the bacillus of Koch.

"(*f*) But this was not invariably the case. At least a careful search failed to demonstrate the presence of this bacillus in tubercles found in the lungs of three rabbits (half-grown white rabbit, killed October 8, Experiment No. 3; large, spotted rabbit, killed October 7, Experiment No. 4; small rabbit killed by subcutaneous injection of human saliva, October 6, Experiment No. 5).

"(*g*) The bacillus of Koch was found in abundance in the tubercular nodules from a freshly cut section of human lung, obtained through the courtesy of Prof. Hirschfelder, from the dead-house of the City and County Hospital, San Francisco.

"(*h*) But in a similar specimen from another case, repeated examination failed to demonstrate the presence of the bacillus.

"(*i*) Culture-experiments have demonstrated that the bacillus in question multiplies upon the surface of sterilized and jellied blood-serum, prepared in the manner described by Koch.

"(*j*) But in the writer's experiments the bacilli have not penetrated the culture-medium, or extended upon its surface to such an extent as to indicate that multiplication was at the expense of this jellied blood-serum, which has seemed rather to serve as a *moist*, supporting surface; multiplication has apparently been at the expense of the tubercular material introduced for the purpose of inoculating this culture-medium. The bacilli were not abundant in this material when it was first obtained from the lungs, or from an enlarged lymphatic gland of a rabbit recently killed; but after remaining in the culture-tube for a fortnight the bacilli were found in enormous numbers, while the cellular elements of the tuberculous material had to a great extent disappeared."

Dr. Feltz of Nancy reports that his attempts to cultivate the tubercle bacillus have been entirely unsuccessful, although he claims to have exactly followed the directions given by Koch in his original paper.

It will be seen from the above quotation that the writer's attempt to cultivate the bacillus was also, practically, a failure; for it only multiplied in the tubercular material, and consequently could not be isolated from it.

If these experiments do not give strong support to the view that the bacillus bears a causal relation to the disease, they are at least not entirely opposed to this view; and it must be admitted that they can be accorded but little weight as opposed to the uniformly positive results reported by Koch in a large number of inoculation experiments, and the strong confirmation which he has received from Cheyne, a very competent witness in a case of this kind.

This author paid a visit to Toussaint and to Koch for the purpose of making himself familiar with their methods. Upon his return to England, a series of experiments was made, with the results reported below.

The experiments were made under the most favorable hygienic conditions, and all possible precautions were taken as regards disinfection of instruments and the complete isolation of the animals used. Twenty-five animals, inoculated with non-tubercular material in various ways, failed to become tuberculous. In six of these, setons were introduced subcutaneously; in ten, vaccine lymph was employed; in three, pyæmic pus was injected; and in six, various materials were introduced into the abdominal cavity (cork, tubercle hardened in alcohol, worsted thread). Cheyne believes that in similar experiments made by other observers, in which a positive result has been reported, the tubercle bacilli have always been introduced accidentally, with the innocuous material to which the result has commonly been ascribed.

Toussaint, who has ascribed the disease to a micrococcus, furnished our author cultures of this micrococcus obtained by inoculating blood-serum or rabbit *bouillon* with the blood of a tuberculous animal. This material was injected into three rabbits, two guinea-pigs, one cat, and one mouse. In no instance did tuberculosis ensue. The injections in Cheyne's experiments were made, whenever practicable, into the anterior chamber of the eye, with a syringe which had been purified by heat. Cultivations of the micrococci obtained from Toussaint were also made and injected into nine rabbits and three guinea-pigs, with a negative result. The tuberculous organs of animals experimented upon by Toussaint were examined by Cheyne, who found in them, often in large numbers, the bacillus of Koch, but no micrococci; although some of these animals had developed tuberculosis as a result of inoculation by Toussaint, with cultures of the micrococcus described by him. This result is ascribed to accidental inoculation with the spores of the tubercle bacillus, which Cheyne shows would not be destroyed by the method of disinfection upon which Toussaint has relied, namely, the cleansing of his syringe with an aqueous solution of carbolic acid.

Twelve rabbits were also inoculated with cultivations of the tubercle bacillus obtained from Koch.

"*All of these became tuberculous, and that more rapidly than after inoculation with tuberculous material.* The tubercles produced in these cases were infective and produced tuberculosis in other animals. On examination of tuberculous material, Koch's bacilli are *always* found, though in varying numbers. They are most numerous in bovine tuberculosis, and least numerous in human tuberculosis. About eighty organs of tuberculous animals and thirty-six cases of human tuberculosis were examined, and in all of these, without exception, tubercle bacilli were found."[1]

TYPHOID FEVER.—The established facts relating to the origin of isolated cases and local epidemics of typhoid fever all point to the existence of a *contagium vivum* capable of self-multiplication external to the human body, and which commonly gains access to the intestinal canal of those attacked with the disease through the ingestion of infected material, and especially of unboiled fluids, particularly of water and milk. It is generally recognized that the infective agent is contained in the stools of typhoid patients, and that it may increase indefinitely in a proper pabulum, and under favorable conditions as to temperature, when these stools are carelessly mixed with organic material in cess-pools, privy-vaults, etc.

There is nothing in the clinical history of the disease under consideration, or in known facts relating to its epidemic extension, to indicate that the typhoid germ multiplies in the blood of those attacked with the disease; and the negative results which have, for the most part, been reported by those who have sought it in this fluid, correspond with what might *a priori* have been expected.

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[1] Quoted from abstract in "Braithwaite's Retrospect," Part LXXXVII. p. 73.

Meyer, however, has reported the finding of bacilli in great numbers in the blood of a case of typhoid which resulted fatally from congestion of the lungs and kidneys, at the end of two days. But it may be questioned whether the pathological appearances would be sufficiently marked at so early a date to establish the diagnosis; and, in any event, the finding of micro-organisms in blood obtained *post mortem* has little import, unless the same organisms were found in this fluid before death. Almquist reports that he has occasionally found groups of *microbes* in the blood in small numbers. These were short rods, and were most abundant during the second or third week of sickness. In this, as in other diseases, we must bear in mind the possibility that a septic complication may be attended by invasion of the blood by micro-organisms not bearing any direct relation to the typhoid process; and also that non-pathogenic bacteria may possibly invade the circulating fluid when the vital powers are at a low ebb.

Maragliano found in blood drawn from the spleen by means of a hypodermic syringe, motile and motionless micrococci, and also a small number of rods like those described by Eberth. Letzerich also claims to have recognized the micrococci which he supposes to be the specific germs of typhoid in the blood and in the sputum. Moxon, in a recent paper "On our Present Knowledge of Fever," remarks as follows: —

> "You must not suppose that one has only to get a microscope and a slide and put a little fever blood under it to find it full of germs. No; try in any of our cases of typhoid in the wards, and you will find these germs by no means very easily discovered or obvious things. At the outset of such an inquiry, you must take notice that the blood-serum is often crowded with minute particles, which must not be confounded with bacteria, and which exist, often to a large extent, in the blood of healthy persons. During last winter's clinical session, some of my most acute and intelligent friends searched carefully for germs in the blood of several severe typhoid cases. The result was that one bacterium was seen, only one, but I was told it was a very active one. When I say that Mr. Booth saw it, you will know it was well seen, for we all regard Mr. Booth as one of the very ablest and very best students at Guy's; but perhaps the main fact was that all were quite sure that there was only one bacterium."[1]

The attempts which have been made to produce typhoid fever in the lower animals have not given any results of a sufficiently definite character to make it possible to study the etiology of this disease by the method of inoculation with pure-cultures of suspected organisms. And for the present the evidence in favor of the various organisms which have been supposed by different observers to be the veritable typhoid germs, is mainly that obtained by the microscopical examination of the tissues involved in the local lesions which characterize the disease. When we consider that the healthy intestine is the usual habitat of a large number of species of bacterial organisms, and that some of these promptly invade necrotic tissues, — and possibly living tissues having a low grade of vitality, or which are deprived of their normal relations by inflammatory exudates which furnish a suitable pabulum for parasitic micro-organisms, — we shall appreciate the difficulty of deciding whether necrosis of invaded tissues is a result of the parasitic invasion, or whether the mycosis has been secondary to and independent of the morbid process.

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[1] Lancet, December 9, 1882, p. 974.

Eberth seems to have very fully appreciated these difficulties, and it is doubtful whether any more satisfactory evidence can be obtained than that which he has offered in favor of the view that the bacillus described by him is the much sought typhoid germ, unless future experiments upon the lower animals give more definite results than have been heretofore reported. As pointed out by Eberth, the results reported by Walder in his experiments upon calves, dogs, cats, rabbits, and chickens, are entirely unreliable, as no account seems to have been made of septicæmic complications which could scarcely fail to occur from the ingestion of putrid material, — blood, typhoid stools, etc., used in many of his experiments. Letzerich also seems to have been ignorant of the fact that the sputum of healthy persons produces septicæmia in rabbits, and his inference that rabbits inoculated with the sputum of a fever patient suffered an attack of genuine typhoid, is probably as wide of the mark as was Pasteur's with reference to the "new disease" described by him as resulting from inoculating rabbits with the saliva of a child dead of hydrophobia. One of Letzerich's rabbits died at the end of five days, and one was killed at the end of twelve days. Micrococci and rods were found in the spleen, in the veins, and in the follicles of the intestine, but the evidence presented in favor of the view that these animals had typhoid fever is entirely unsatisfactory.

Brantlecht produced in young rabbits most of the typhoid symptoms by the subcutaneous injection of culture-liquids; but he obtained the same results with bacilli found during the summer months in the water of stagnant ponds (Cameron). Chomjakoff, a pupil of Klebs, injected typhoid bacilli (?) into the peritoneum of rabbits. The animals immediately exhibited an elevation of temperature, which attained its maximum on the third day. They all died on the third or fourth day, in two instances with diarrhœa. The lesions were, redness and tumefaction of Peyer's glands, increase in volume of the spleen, cellular infiltration of the intestinal tissues. The presence of micrococci was doubtful, but the peritonitis was in inverse ratio to the cultivation. The evident criticism in experiments of this kind is that the results are necessarily complicated by the peritonitis resulting from the introduction of micro-organisms into the cavity of the abdomen; that the symptoms follow the injection immediately, while in man there is a certain period of incubation; and that the death at the end of four days of an animal very susceptible to various forms of septicæmia, but which, so far as we know, never contracts typhoid fever spontaneously, can hardly be taken as evidence that the micro-organisms injected into its peritoneal cavity were veritable typhoid germs. Indeed, we cannot help suspecting that other investigators operating with micro-organisms from other sources would have found in the symptoms and pathological lesions evidence of yellow fever, or of continued malarial fever, or possibly of scarlet fever, without the rash; for the absence of the characteristic rash could be easily explained by the fact that the integument is thickly covered from sight by a heavy growth of hair. Klebs also introduced cultivated typhoid organisms into the cavity of the abdomen in eleven rabbits. In one only death occurred at the end of four days; and upon this slim foundation the inference was made that the organisms used in the experiment were veritable typhoid germs.

"Tigri first found bacteria in the blood of a man dead with typhoid fever. These organisms were also found by Signol (1863) and Mégnin (1866) in the blood of horses attacked by a disease called by the veterinarians *typhoid fever.* This blood, by inoculation, produced the death of some rabbits, with the same alterations in the blood.

"Coze and Feltz (1866), having inoculated some rabbits with the blood of typhoid fever, have produced results which they consider analogous, and as accompanied by the same pathological localizations in the glands of Peyer. The blood of an injected rabbit may be used upon a second rabbit, with positive results, as in variola and scarlatina.

"The species of *Bacterium* which is found in this case recalls the *Bacterium catenula*, but its dimensions are less." (Magnin.)

The presence of micro-organisms in the local lesions of typhoid fever has been verified by numerous observers, and, as already remarked, was *a priori* to have been expected. The statistics of these observations, therefore, which Eberth has given us, although interesting, have comparatively little value. According to this author, Von Recklinghausen first described micro-organisms in abdominal typhus.

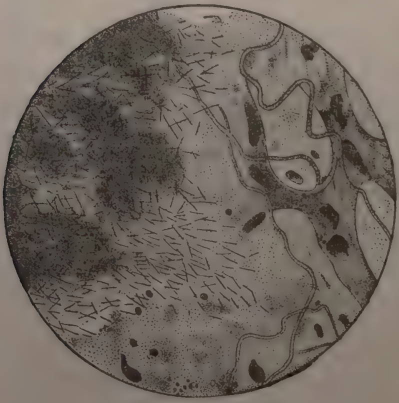

Fig. 23.

Vertical section of intestine, typhoid fever, showing the border of the submucosa infiltrated by bacilli. Hartnack im. No. 9, Ocular 2 (Klebs).

These were found in the typhoid ulcers, and consisted of masses of micrococci. Klein also found groups of micrococci in the mucous membrane, in the lymph follicles, and in the spleen. Fische found colonies of micrococci in the spleen and in the lymphatic glands in fifteen out of twenty-nine cases examined. The positive results were mostly obtained in recent cases; some of these, however, gave a negative result. Klebs found organisms — micrococci or bacilli — in twenty-four cases examined by him. Koch found bacteria in half the cases which he examined; Meyer in eighteen out of twenty-four; and Eberth in eighteen out of forty. Eberth remarks that the result would probably have been more favorable but for the fact that the organism in many cases seems to have been destroyed in the tissues. In the negative cases the height of the fever was already past. The bacilli are said to be most numerous during the first twelve or fourteen days of sickness, less numerous at the end of the third week, and they were seldom met with in the fifth

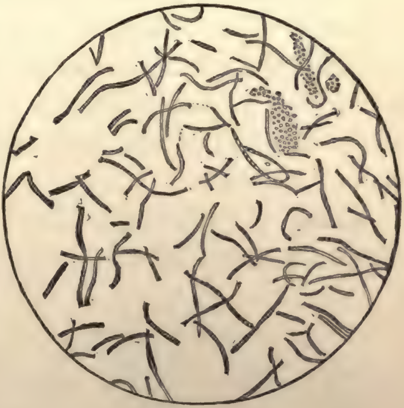

Fig. 24.

From a fresh section of typhoid intestine; treated with glacial acetic acid and glycerine mixture. Siebert's im. No. 7, Ocular 3 (Klebs).

or sixth week; if found then they present evidence of having undergone retrograde change.

The typhoid germ of Letzerich is a micrococcus, isolated, in colonies, or in chains, very dissimilar to those of diphtheria and of infectious pneumonia, but which by cultivation may reach twice or three times the size of the micrococci of the last mentioned diseases.

Klebs describes his *Bacillus typhosus* as large-sized filaments of 50 μ in length and 0.2 μ in breadth, without segments or ramifications. When the spores make their appearance the filaments may reach 0.5 μ in breadth. The spores are arranged in a line, and very close together. Before they are formed, the bacilli exist as short rods (see Figs. 23, 24, and 25).

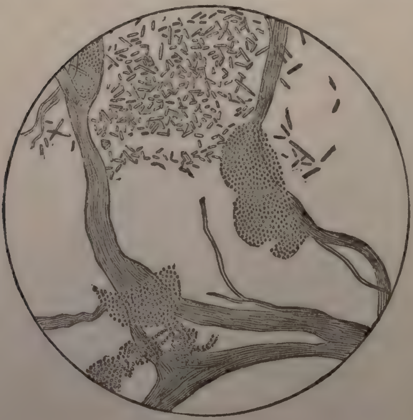

Fig. 25.

Section of typhoid lung; fresh; treated with mixture of glycerine and glacial acetic acid. Siebert's im. No. 7, Ocular 3 (Klebs).

The morphological characters of the bacillus of Eberth are shown in Fig. 26, which is copied from his paper, referred to in bibliography.

When these bacilli are present in great numbers they have the appearance of masses of micrococci.

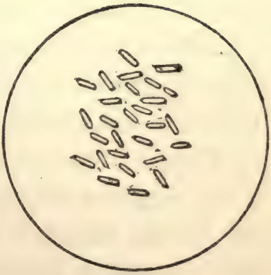

Fig. 26.

Typhoid bacilli from a lymphatic gland. Hartnack No. 12, Ocular 3. (From Eberth, "Der Typhus-bacillus und die intestinale Infection.")

But when isolated from these masses they are recognized as short thick rods having rounded ends. With high powers many of the bacilli may be seen to contain two or three granules, which are probably spores. The rods are sometimes found, in the juice scraped from the freshly cut surface of a diseased lymphatic gland, in chains of two or three elements. The characters by which these bacilli are recognized are the rounded extremities, and the fact that they are not so deeply stained by the aniline dyes as are the putrefaction bacteria often found in the same preparation. In addition to these bacilli, Eberth recognizes at least seven micro-organisms which he has met with in his microscopical studies, and which may be associated with them. But the bacillus with rounded ends is said to be peculiar to typhoid, and has not been found in a single instance out of twenty-four cases of intestinal disease of a different character, — e. g., tuberculosis of the bowels, — in which he has made a careful examination by the same methods. Similar negative results were obtained by Mayer in six cases of dysentery and other diseases of the bowels. Koch is of the opinion that the bacillus of Eberth is the only one which has a specific relation to the disease. According to this observer Klebs's elongated bacilli belong to the putrid parts, and only invade the necrotic tissues which have succumbed to the attack of the specific typhoid bacillus.

Eberth also describes a small and comparatively long bacillus, which no doubt corresponds with that of Klebs, which is found isolated and in groups *in the superficial layers of the necrotic tissues.* "Their appearance and color-reaction show them to be ordinary putrefaction bacteria of the intestinal contents." As evidence of the number of bacterial organisms constantly present in the intestinal canal of healthy persons, the reader is referred to the photo-micrograph in Plate VII., Fig. 4. This, however, by no means shows all the forms which may be found at different times in the discharges of persons in perfect health. (See also Plate XIII., illustrating the writer's paper on "Bacteria in Healthy Individuals" in Vol. II., No. 2, of "Studies from the Biological Laboratory" Johns Hopkins University.)

Coates, of Glasgow, confirms Eberth as to the presence of the bacillus described by him in a diseased lymphatic gland removed from a case of typhoid fatal on the ninth day. Crook has also found the bacillus in a case treated in the Fever Hospital of Leeds.

The writer would simply remark, in regard to this bacillus, that the distinctive character upon which Eberth chiefly relies, seems hardly sufficient to establish it as a distinct species, when we compare his figure (Fig. 26) with that of Cheyne (Fig. 28), and with my photo-micrograph, Fig. 1, Plate VIII. Certainly the rounded ends of this typhoid bacillus are not peculiar to it.

ULCERATIVE ENDOCARDITIS.—"In this affection, it is well settled to-day that the cardiac walls and, above all, the valves, are covered with parasitic masses. Some think that the malady is due to the introduction of these parasites into the interior of the tissues; others, on the contrary, like Hiller, deny that the bacteria bear any causal relation with the lesions of ulcerative endocarditis." (Magnin.)

VARIOLA.—"The partisans of the parasitic nature of variola may be divided into two groups: 1. Those who, with Coze and Feltz, attribute the virulence to a *Bacterium;* 2. Those who, with Luginbühl and Weigert, attribute it to a *Micrococcus.* Coze and Feltz have indeed discovered bacteria in the blood of variola, and this blood injected into the veins of a rabbit has given it a mortal malady, which these observers consider variola. But Chauveau has shown that the affection which proved fatal to the subjects of the experiment was not and could not be variola. Another objection is that bacteria are not found in all those who suffer from variola. However, Coze and Feltz and Baudouin affirm that there are in variolous blood numerous rods, of which the appearance is similar to that of *Bacterium bacillus* and *Bacterium termo* of Müller. These elements do not at all resemble those found in other infections, and when inoculated possess the power of reproducing variola.

"As to the *Micrococcus* of variola, they have been studied by Luginbühl, Weigert, Hallier, and Cohn. These micro-organisms possess the characters of all the spherical bacteria, and are found in the variolous pustules, the *rete Malpighii*, the liver, the spleen, the kidneys, and the lymphatic ganglia. We can only insist upon the fact of the concomitance of the variola and the presence of micrococci, since experiment cannot be resorted to in this disease, of which the complete evolution occurs only in man. We also find in vaccine lymph micrococci analogous, in every point of view, to those of variola. Cohn considers them both, not as distinct species, but as two races of the same species, — the *Micrococcus vaccinæ*." (Magnin.)

"M. Straus presented to a recent meeting of the Société de Biologie at Paris a series of microscopical preparations of the vaccinal pustule of the calf, at different stages of its progress, in which the presence of the special micrococcus could readily be observed. The method of preparation adopted was to place the excised fragments of skin in absolute alcohol, to cut sections, and stain by Weigert's method (methylamine violet), and then discoloring them until only the nuclei, the bacteria, and micrococci remain visible. Under a high power, the latter were visible as extremely minute points, tinted blue, about a thousandth part of a millimeter in diameter, and grouped in colonies. They were seen in the borders of the inoculation wound, and in the Malpighian layer, and subsequently could be traced passing into the subjacent cutis, especially in the lymphatic spaces. The multiplication and extension of the organism seemed to coincide closely with the development of the pustule."[1]

Dr. Wolff claims to have successfully cultivated the *micrococcus vaccinæ* through fifteen successive generations.[1] If this is true he will be able to claim the prize offered by the Grocers' Company of London: —

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[1] J. Roy. Microscopical Soc., Oct. 1882, p. 661.

> "The subject of the Grocers' Company's first discovery prize of £1,000 for original research in connection with sanitary science is 'A method by which the vaccine contagion may be cultivated apart from the animal body, in some medium or media not otherwise zymotic; the method to be such that the contagium may by means of it be multiplied to an indefinite extent in successive generations, and that the product after any number of such generations shall (so far as can within the time be tested) prove itself of identical potency with standard vaccine lymph.' The prize is open to universal competition, British and foreign. Competitors for the prize must submit their respective treatises on or before the 31st of December, 1886, and the award will be made as soon afterwards as the circumstances of the competition shall permit, but not later than the month of May, 1887. All communications on the subject must be addressed to the clerk of the Grocers' Company, London, from whom circulars giving the conditions can be obtained."

VARIOLA OF PIGEONS. — In a communication to the French Academy, presented by Vulpian, M. Jolyet gives an account of an experimental research, made in collaboration with MM. Delâge and Lagrolet, relating to the etiology of the disease known as variola of the pigeon or *picote*. He says: —

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[1] Berlin Klin. Wochenschrift, Jan. 22, 1883.

"Microscopical examination of the blood of pigeons attacked with variola shows that this liquid contains an infinite number of living microbes. This alteration is constant, and is true in the case of pigeons attacked spontaneously, as well as of those which have been subjected to experimental inoculation.

"Upon studying the development of the microbes in the blood, the following facts worthy of note may be observed. The first important point consists in the progressive development of the organisms in correspondence with the progress of the disease. Their appearance in the blood always precedes the appearance of morbid phenomena. This fact is especially easy of verification in pigeons which have been inoculated, by means of a vaccination needle, either with the blood of a sick animal, or with the liquid contained in the pustules.

"If after inoculation we examine each day the blood of pigeons, we shall find that during the first, second, and often the third day, it presents nothing abnormal in its appearance; however, towards the end of the third day an attentive examination will already demonstrate the presence of the microbes in the blood; the following days the parasite increases rapidly, and when the pigeon presents manifest symptoms of illness, a microscopic preparation of the blood offers myriads of microbes in movement.

"This period, from the time of inoculation until the development of morbid phenomena, corresponds with the period of incubation so characteristic of other virulent and contagious maladies. The greatest number of parasitic organisms are found in the blood just before the eruption appears. Subsequently they gradually decrease in number.

"The pus of the pustules contains the characteristic microbes in abundance, and produces the disease when inoculated into healthy pigeons. . . .

"In a certain number of pigeons the cutaneous eruption is wanting, and in this case the autopsy reveals a veritable intestinal pustulation.

"The microbes from the pustules or from the blood, cultivated in pigeon *bouillon*, have furnished successive culture-liquids which, when inoculated, reproduce the disease.

"But it is the blood (*in vitro*) and the lymph which are the best culture media for the microbes of variola, either of man or of the lower animals. And nevertheless, if we examine the blood of subjects attacked with variola (man, the pig) we find that it contains but few microbes, so that it is difficult to suppose that these organisms are the first cause of the malady. So also in charbon, in many animals but few *bactéries* are found in the blood at the moment of death. This is because, in the living animal, the most favorable medium for the development of these infectious organisms is the lymph. Numerous observations enable us to affirm this fact. . . .

"In conclusion we will say that if the microbes in the course of an infectious malady do not multiply in the blood in circulation, they are susceptible of multiplication in the blood in repose, drawn directly from an artery into Pasteur's flasks — sterilized, and that they retain their specific qualities."

WHOOPING COUGH. — "Poulet, in 1867, found certain bacteria of a peculiar kind in the sputa of patients affected with pertussis; Letzerich commenced a series of investigations a few years later. The latter found constantly present in the sputum of pertussoid patients a bacterium belonging to the genus Ustiligo, Tul.; with this he inoculated the tracheal mucous membrane of tracheotomized rabbits and noted the results. He invariably produced a spasmodic catarrhal affection resembling whooping-cough, and he observed that the bacteria do not penetrate the epithelium, but live on the surface of the mucous membrane, to the detriment of the latter.

"Tschamer, of Gratz, working in the same department of micro-pathology, has lately found, in the expectoration of pertussis, a microphyte, which he identifies with a black mould which develops on orange-peel. This he thinks that he has proved by different cultures. Satisfied of the identity, he took some of the black powder which constitutes the mould of orange-peel and experimented with it on himself, inhaling the powder as deeply as he could. At first no effect was observed, but after eight days he began to have convulsive fits of coughing, and expectorated the fungus in abundance.

"He explains the phenomena of whooping-cough in this way. After an incubation of seven days, these microphytes determine an irritation of the bronchi which induces catarrh and spasmodic cough; then, as the irritation increases, the expectoration becomes more abundant and eliminates the fungoid organisms.

"Dolan, in repeated experiments, found that by inoculating rabbits with the sputum of whooping-cough patients, he not only induced a catarrhal spasmodic affection, *but the death of the animal generally ensued.* Inoculation with the blood of such patients was without effect. This certainly seems to confirm the conclusions of Letzerich, that the *materies morbi*, — be it a bacillus, or be it what it may, — lives on the surface of the epithelium, and does not get into the blood."[1]

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[1] The Medical Record, February 17, 1883, p. 185.

The writer has italicized the sentence in which the editor of the "Medical Record" has incidentally remarked that the death of the animal generally ensues, and would respectfully call attention to his experiments relating to a fatal form of septicæmia in rabbits resulting from the subcutaneous injection of the saliva of healthy individuals.

YELLOW FEVER. — In a paper contributed to the American Journal of the Medical Sciences (April, 1873), the writer has stated the *a priori* argument in favor of the germ theory as regards the etiology of yellow fever in the following language:

"There are three agents, to one of which we must (in the present state of our knowledge) refer the poison, which, by its action upon the human system, produces yellow fever, viz.:

"(*a*) A volatile inorganic matter.

"(*b*) A lifeless organic matter of the nature of a ferment, which, by catalytic action, is capable of transforming otherwise (comparatively) harmless substances, present in the earth or in the atmosphere, into the materies morbi of yellow fever.

"(*c*) A living germ, capable, under favorable conditions as to heat, moisture, etc., of rapid self-multiplication, and acting, either directly, or indirectly by catalytically transforming other substances into the efficient cause of the disease.

"That the poison is of the latter nature, is, I conceive, the only theory consistent with the observed facts in regard to the origin and propagation of the disease, and upon it all the otherwise contradictory facts are reconcilable. In support of this I will first submit a few concise propositions which seem to me capable of proof, and will then briefly discuss these propositions, and the legitimate inferences to be drawn from them:

"1. *The yellow fever poison is not an emanation from the persons of those sick with the disease.*

"2. *It is not generated by atmospheric or telluric influences.* A certain elevation of temperature is, however, necessary for its multiplication; and its rapid increase is promoted by a moist atmosphere, and probably by the presence of decomposing organic matter.

"3. *The poison is portable in ships, goods, clothing, etc., and a minute quantity is capable of giving rise to an extensive epidemic.*

"4. *Exposure to a temperature of* 32° *Fahrenheit completely destroys it.*

"5. *It may remain for an unknown length of time in a quiescent state*, when not subjected to a freezing temperature, or exposed to the conditions necessary to its multiplication, *and may again become active and increase indefinitely when those conditions prevail.*

"If the first three propositions be proven, viz., that the poison is portable, that a small quantity may increase indefinitely, independently of the human body, and that it is not produced by atmospheric influences, then the necessary inference is, that it is capable of self-multiplication, which is a property of living matter only."

The propositions above stated were supported, in the paper referred to, by facts observed during a local epidemic, which occurred on Governor's Island, New York harbor, during the summer of 1870. Other local epidemics, since observed by the writer, and the recorded facts relating to numerous outbreaks of limited extent, and to the extended epidemic in the United States in 1878, followed by a reappearance of the disease in Memphis in 1879, strongly support these propositions, and the inference drawn from them as to the nature of the yellow fever poison. It will be seen, however, that our propositions, if accepted as proven, do not necessarily lead us to the conclusion that the yellow fever germ multiplies within the bodies of those sick with the disease. On the other hand, if the first proposition is true, it seems altogether probable that it does not multiply within the bodies of the sick, but that the poison is evolved as a result of its vital activity during the decomposition of the dead organic material which serves as pabulum for its growth. The observed facts relating to the epidemic prevalence of the disease indicate that decomposing *animal* matter furnishes a suitable nidus for the germ, and consequently the dead body of a yellow fever patient should constitute such a nidus, even if the living body does not. As a matter of fact, infection has very frequently been traced to dead bodies, whereas there is abundant evidence to show that persons contract yellow fever by exposure in infected localities, and *not by contact with those sick with the disease.* Bedding charged with organic emanations from the body of a sick person is also a suitable nidus for the germ. But the infectious character of infected bedding seems to be acquired in infected localities rather than to be due to infection by sick persons. A statement of the evidence which has led the writer to this conclusion would be out of place in the present volume, and without further remark we must proceed to consider the experimental evidence in favor of our *a priori* reasoning. It must be admitted that this is very unsatisfactory.

The writer's personal investigations are recorded in the "Preliminary Report of the Havana Yellow Fever Commission of the National Board of Health," extracts from which report are given below. Unfortunately, the time allotted to this investigation — three months — was entirely too short to make a thorough experimental study; and much of this valuable time was necessarily consumed in perfecting methods of research, and in gaining a knowledge of micro-organisms encountered on every side *which were not yellow fever germs*, but which could not be excluded from consideration until this fact was demonstrated.

Evidently an extended acquaintance with the bacterial organisms found during life and after death in the bodies of persons not suffering from yellow fever, and familiarity with the most approved methods of isolating and cultivating these organisms, would have been of great advantage to the investigator. But this preliminary knowledge and special training was of the most imperfect character. It was therefore evident that unusual scientific caution would be required to compensate, as far as possible, for a lack of previous special preparation for the work in hand; and to avoid the announcement of pseudo-discoveries which, when heralded by an enthusiastic but ignorant explorer, are sure to pass current for a time, inasmuch as a majority of the profession find no time for personal investigations, and do not realize the ease with which an explorer in this field of investigation may fall into a serious error.

Extracts from Report of Havana Commission.

"In Havana, Dr. Sternberg gave a large share of his time to the microscopic examination and photography of the blood. No chemical examination was attempted. The patients from whom specimens of blood were obtained were mostly soldiers in the military hospital of San Ambrosio. Ninety-eight specimens from forty-one undoubted cases of yellow fever were carefully studied, and one hundred and five photographic negatives were made, which show satisfactorily everything demonstrable by the microscope. These photographs were mostly made with a magnifying power of 1,450 diameters, obtained by the use of Zeiss's one-eighteenth-inch objective and Tolles's amplifier. Probably no better lens than the Zeiss one-eighteenth (oil immersion) could have been obtained for this work, and it is doubtful whether any objective has ever been made capable of showing more than is revealed by this magnificent lens. With the power used, organisms much smaller than those described as existing in the blood of charbon or of relapsing fever would be clearly defined.

"If there is any organism in the blood of yellow fever demonstrable by the highest powers of the microscope as at present perfected, the photo-micrographs taken in

Havana should show it. *No such organism is shown in any preparation photographed immediately after collection.* But in certain specimens, kept under observation in culture-cells, hyphomycetous fungi and spherical bacteria made their appearance after an interval of from one to seven days. The appearance of these organisms was, however, exceptional, and in several specimens, taken from the same individual at the same time, it occurred that in one or two a certain fungus made its appearance and in others it did not. This fact shows that the method employed cannot be depended upon for the exclusion of atmospheric germs, but does not affect the value of the result in the considerable number of instances in which no development of organisms occurred in culture-cells in which blood, in a moist state, was kept under daily observation for a week or more.

"The method employed seemed the only one practicable for obtaining blood from a large number of individuals without inflicting unwarrantable pain and disturbance upon the sick. It was as follows: One of the patient's fingers was carefully washed with a wet towel (wet sometimes with alcohol and at others with water) and a puncture was made just back of the matrix of the nail with a small triangular-pointed trocar. As quickly as possible a number of thin glass covers were applied to the drop of blood which flowed, and these were then inverted over shallow cells in clean glass slips, being attached usually by a circle of white zinc cement. In dry preparations, which are most suitable for photography, the small drop of blood was spread upon the thin glass cover by means of the end of a glass slip.

"The thin glass covers were taken from a bottle of alcohol and cleaned immediately before using, and usually the glass slips were heated shortly before applying the covers, for the purpose of destroying any atmospheric germs which might have lodged upon them. These precautions were not, however, sufficient to prevent the inoculation of certain specimens by germs floating in the atmosphere (Penicillium spores and micrococci); and in nearly every specimen the presence of epithelial cells, and occasionally of a fibre of cotton or linen, gave evidence that under the circumstances such contamination was unavoidable. It is therefore believed that any organism developing in the blood of yellow fever, or of other diseases, collected by the method described, or by any similar method, can have no great significance unless it is found to develop as a rule (not occasionally) in the blood of patients suffering from the disease in question, and is proved by comparative tests not to develop in the blood of healthy individuals, obtained at the same time and by the same method.

"Tried by this test it must be admitted that certain fungi and groups of micrococci, shown in photographs taken from specimens of yellow fever blood collected at the military hospital and preserved in culture-cells, cannot reasonably be supposed to be peculiar to or to have any causal relation to this disease. While we can claim no discoveries from the microscopic examination of the blood, bearing upon the etiology of yellow fever, some interesting observations have been made relating to the pathology of the blood in this disease.

"It is not intended in this report to do anything more than make a brief reference to these observations, as a comparative study of the blood of other diseases will be required to give value to them, and a detailed report upon this subject is to be made at some future time. The most important observation made relates to certain granules in the white corpuscles shown in many of the

PLATE XII.

Fig. 1. — Blood from finger of yellow fever patient in Military Hospital, Havana, 1879 ; fifth day of sickness ; fatal case. × 400 diameters by Beck's $\frac{1}{5}$ inch objective.

Fig. 2. — Blood from finger of yellow fever patient in Military Hospital, Havana, 1879 ; fifth day ; fatal case. × 1450 ; Zeiss's $\frac{1}{18}$ inch hom. oil im. objective.

Fig. 3. — White blood corpuscle from yellow fever blood of fifth day, showing fat granules. × 1450.

Fig. 4. — White blood corpuscle from yellow fever blood of fifth day, showing fat granules. × 1450.

PLATE XII.

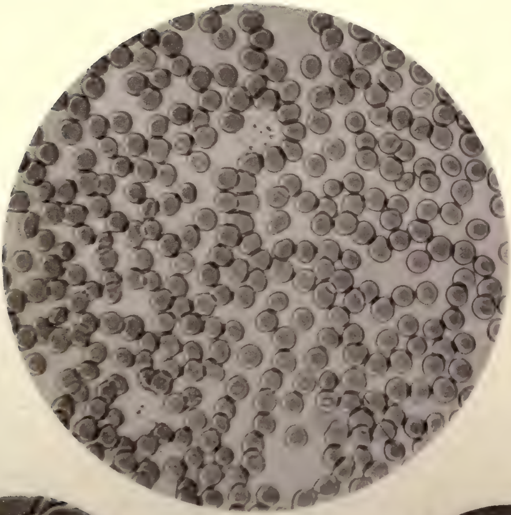

FIG. 1.

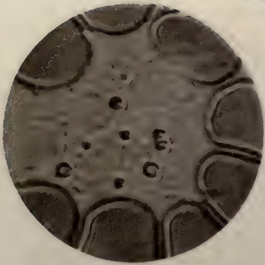

FIG. 3.

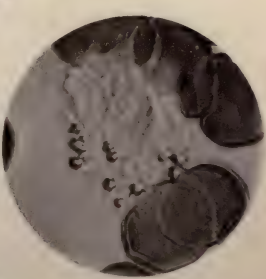

FIG. 4.

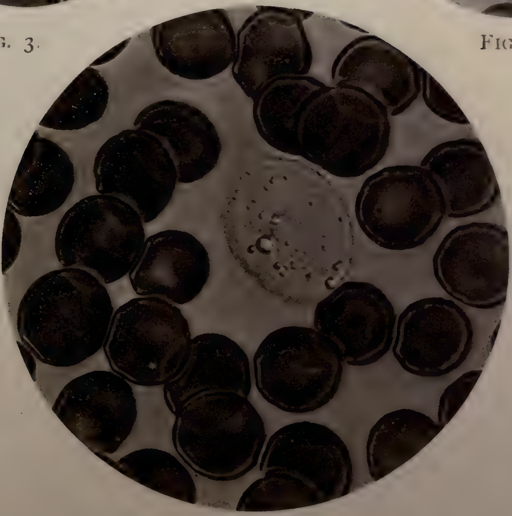

FIG. 2.

photo-micrographs taken. From the manner in which these granules refract light, and for other reasons, they are believed by Dr. Sternberg to be fat, and to represent a fatty degeneration of the leucocytes.

"The blood of twelve healthy individuals was examined in Havana for comparison, and in nearly every case an occasional leucocyte was found to contain a few (one or two) granules undistinguishable from those found in the blood of yellow fever; but this was the rare exception; while in severe cases of yellow fever the granules were abundant, and nearly every white corpuscle contained some of them."

The granules referred to are well seen in the heliotype reproductions of the writer's photo-micrographs made in Havana. (See Figs. 1, 2, 3, and 4, Plate XII.)

Upon comparing the granules referred to, as seen in Fig. 3, Plate XII., with a photo-micrograph of the spores of bacilli (Fig. 3, Plate VIII.) made with the same amplification, a very striking resemblance will be noticed. Indeed it would be impossible to determine from the optical appearances alone that in one case we are dealing with fat-granules, and in the other with reproductive spores. The size and the refractive index are the same, or very nearly so. These granules were new to the writer when he first encountered them in the blood of yellow fever patients, and it seemed not improbable that a discovery of value had been made. Much time was accordingly given to their study. The result of this was to convince the writer that they were fat-granules, probably developed in the leucocytes, and representing a fatty degeneration of their protoplasm, but possibly picked up from the blood. In the white corpuscle in the centre of Fig. 2 it will be noticed that these granules are of various sizes, and that they do not so closely resemble bacillus spores. The conviction that they were really fat-granules was not reached, however, until after a protracted study of yellow fever blood, enclosed in germ-proof culture-cells, which admitted of frequent microscopical examination of their contents. In these cells no evidence was obtained that these granules increase by fission or grow into rods, as we should expect if they were reproductive bodies. On the other hand, they increased in size, became diffluent, and after a time the leucocyte presented the appearance of having been resolved into a little collection of oil globules.

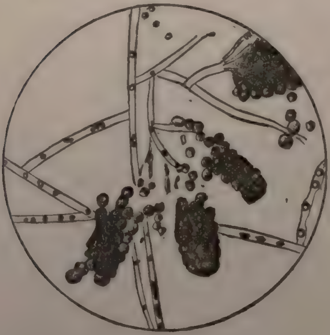

Fig. 27.

Penicillium from culture-cell containing blood of yellow fever patient. × 200. (From photo-micrograph, Havana, 1879.)

The inference that the species of *Penicillium* (see Fig. 27) which not infrequently appeared in my culture-cells was developed from air-borne spores which accidentally fell upon the drop of blood during the brief period required for hermetically enclosing it, and not from spores present in the blood prior to its withdrawal from the body, was probably correct. But it must be admitted that the argument offered in favor of this view has no great weight, and that the inference may be a mistake. The fact that the fungus only appeared occasionally in my culture-cells would be quite easily reconciled with its somewhat abundant presence in the blood; for an organism of this size might be present in considerable numbers without being found in every drop drawn from the finger. But direct examination of very many specimens of blood did not show it, whereas it is well known that the spores of *Penicillium* are among the most numerous of the organized particles suspended in the atmosphere; and their abundant presence in the air of the Military Hospital of Havana was demonstrated by aspiration experiments and microscopic examination.

That portion of the Report of the Havana Commission which relates to experiments on animals is here quoted in full, as one of the objects which the writer has had in view in the preparation of the present volume has been to enable those who propose to enter upon experimental investigations of this nature to readily avail themselves of the experience gained by others who have preceded them:

Experiments upon Animals.

"It has been commonly reported, and is asserted by several writers of acknowledged ability, that during the prevalence of yellow fever certain of the inferior animals exhibit symptoms of sickness which are attributable to the influence of the yellow fever poison.

"(Vide Barton, Cause and Prevention of Yellow Fever, third edition, pp. 52–55; Feraud, de la fièvre jaune à la Martinique, p. 271; La Roche on Yellow Fever, Vol. II., pp. 316–318; Blair, Yellow Fever Epidemic of British Guiana, third edition, p. 63.)

"In view of these reports, the Commission was instructed as follows: 'It is obvious that if it be found possible to produce some specific symptoms in some one of the lower animals by exposing such animals in localities known to be capable of producing the disease in man, and thus to establish a physiological test of the presence of the cause of the disease, we may even hope to be able to determine the nature of and the natural history of this cause, although prolonged investigation may be necessary to effect it.'

"The Commission has endeavored to carry out the views of the Board of Health in this direction, but in consequence of the limited time at its disposal, the want of a suitable place to keep the larger animals, and the amount of work in other directions expected from it, it has been found impossible to make an exhaustive experimental investigation. Enough has been done, however, to make it appear highly probable that the sickness and mortality reported among animals during the prevalence of yellow fever epidemics has been improperly ascribed to the influence of the yellow fever poison. It is well known that many of the inferior animals suffer from epidemic diseases peculiar to their several species, and this is especially the case in southern latitudes. We know of no reason why such epidemics should not occur coincidently with yellow fever in man, and it is not surprising that many people unaccustomed to close observation should attribute the sickness in man and in the animals affected to the same cause. In advance of any experiments designed to test the truth of such a deduction, it seemed quite improbable, from the fact that the supposed effect only results exceptionally, if at all, while domestic animals are frequently exposed in large numbers, in localities visited by severe epidemics of yellow fever, without exhibiting any symptoms of sickness. This fact is vouched for by many competent observers, and is verified by the personal experience of two members of this Commission.

"Nevertheless, in view of the reports referred to, of the great importance in the prosecution of the investigation of a test of the presence of the poison, and of the possibility that by close observation and the use of the clinical thermometer some symptoms heretofore overlooked might be discovered sufficient to serve as such a test, it was evidently imperative that experiments should be tried in this direction. Arrangements were accordingly made before leaving New York for a supply of animals as required, and on the 24th of July the following were received, per steamer 'Niagara,' viz.: Four dogs, two cats, six rabbits, six guinea-pigs, one monkey, six chickens, twelve pigeons, and two geese. Subsequently (August 30) six more dogs were received.

"All of these animals were carefully observed, and various experiments were tried for the purpose of testing their susceptibility to the influence of the yellow fever poison. The details of these experiments are given in a special report to the National Board of Health, dated October 15. It is not deemed necessary to give these details in the present report, but the general statement may be made that the results were negative. No symptoms were produced in any of the animals experimented upon which can fairly be attributed to the influence of the yellow fever poison.

"The clinical thermometer was constantly used for the purpose of recognizing any slight febrile movement which might possibly occur, and the blood was examined microscopically from time to time. As the experiments made gave no promise of positive results, the Commission did not feel justified in giving more time to this portion of the investigation. It is, however, of the opinion that the reports heretofore referred to, and the importance of a physiological test of the presence of the poison would justify the National Board of Health in pursuing this inquiry in future, especially with such animals as this Commission has not experimented upon. A few experiments are here given as examples of those made:

"*Exp. No.* 1.—On the morning of July 28, four days after arrival in Havana, the following animals were exposed on board the infected brig 'John Welch, Jr.,' viz.: two dogs, two cats, one monkey, two rabbits, three guinea-pigs, two geese, three chickens. The time of exposure was forty-eight hours, at the expiration of which time the animals (in cages) were brought back to the laboratory. The 'Welch' was a very foul ship, and was loaded with molasses. During the time the animals remained on board six of her crew (all) were down with yellow fever. After bringing the animals back to the laboratory, the temperature of each was carefully taken, and daily observations were continued for some time after. No symptoms of sickness presented themselves, except in the case of one dog. This animal suffered a sharp attack of fever, but it is believed that the case was one of a disease common to imported dogs in Cuba, known as *romadizo*, a disease the clinical history of which is very different from that of yellow fever.[1]

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[1] See special report to National Board of Health, dated October 15, for full history of this case.

"*Exp. No.* 4.—Injected yellow fever blood, one and a half drachms, of first day, into femoral vein of dog No. 3. Blood obtained by cupping from patient in civil hospital and mixed with a small quantity of soda bicarb., to prevent coagulation. *Result*, entirely negative.

"*Exp. No.* 10.— One-half of a blanket from a yellow fever patient's bed was placed in the cage with dog No. 4, and left there for several days. *No result.*

"*Exp. No.* 11.—Dog No. 5 was allowed no water for two days, except a supply in which the other half of this blanket (Exp. No. 10) had been washed. *No result.*"

Other experiments were made, in which the blood of yellow fever patients, *obtained post mortem*, was injected into rabbits and guinea-pigs with fatal results. But no importance was attached to these experiments, as several hours had in every case elapsed after the death of the patient before a *post mortem* examination was obtained and the blood collected. It is well known that putrid blood kills rabbits, and also that the blood of scarlet fever and other diseases, obtained *post mortem*, produces death when injected beneath the skin of these animals. Similar results follow the injection of other material containing the bacteria of putrefaction, as shown by the following experiment made in New Orleans at a time when yellow fever was not prevalent:

Exp. No. 13.—October 7, 9 A. M.—Injected into right flank of rabbit 1.35 c. c. of water shaken up with a little material scraped from the surface of gutter-mud in front of my laboratory. The animal was found dead at 8.30 A. M. October 9, and had evidently been dead some hours. *Post mortem* examination shows diffuse cellulitis and gangrenous sloughing of the integument and subjacent tissues of the right side of the belly. So extensive has been this sloughing that the intestines are exposed. A very offensive odor of putrefaction is given off by the gangrenous tissues.

Having reported my own failure to find the yellow fever germ, I must now refer to the recent announcements of its discovery in Mexico by Dr. Carmona, and in Brazil by Dr. Freire. According to the first-named observer, the parasitic element is found in the blood, in black vomit, and in the urine of yellow-fever patients.

The following description is copied from the "Medical News" of July 21, 1883:

> "The general agent wanting in none of these substances is a granular matter, only seen with a microscope of 1,500 diameters, very abundant, ovoid, and slightly yellow, which appeared to have filaments similar to vibrating ciliæ, and having peculiar movements, with a tendency to repeat these again and again. At rare intervals it curls itself in its greater diameter, and generally arranges itself on its side, gradually approximating the extremities until they meet; then it regains its ovoid form, which is similar to that of the prostate gland. These granulations are capable of increasing or maturing, and, under special conditions, gradually lose their first movement, and then unroll themselves into *spherical* bodies of yellow color, uniform aspect and dimensions, eight or ten times larger than the first

granulations. These are from 5 to 12 μ in diameter. These large granulations were those which first attracted attention in the urine of the patients first examined, and since found in the cellular tissues, serum of blisters, and other points of the organism. There were in the urine threads, evidently mycelia, — some so large as to cover the whole field of view, others smaller; and, besides, there were abundant fragments, of various forms and dimensions. Some were more delicate and of a cellular aspect; others more compact and larger, of a brilliant yellow color and of fatty aspect; some of a more reddish color; others emerald green; still others, but much more rare, of a blue color. Their diameters varied from 2 to 20 μ. Cells were frequently encountered completely empty, of rounded or pyriform shape and variable dimensions. *Many of these cells were not entirely empty, but contained a red or yellow granular material*, similar to the points observed in the gold-stone."

The writer has ventured to italicize this description of these partially empty cells, as it recalls to his mind a story told him by his friend, Dr. J. J. Woodward, of the United States Army, whose skill as a microscopist is pretty generally recognized, both in this country and in Europe.

Dr. Woodward states that several years since a distinguished (?) professor from one of the Western cities came to Washington to show him the germ of malarial fever which he had recently discovered. An examination of his specimens showed that the supposed alga (cryptococcus) was nothing more nor less than the little depressions in the surface of the glass slide upon which his material was mounted, filled with the grains of rouge powder used by the manufacturers for polishing these slides. These little crypts, partly filled with grains of red or yellow rouge powder, are very abundant on the surface of some glass slides.

And this recalls a mistake made by the writer soon after his arrival in Havana in 1879. Upon aspirating the air in front of my laboratory through a small aperture, against a thin glass cover smeared with glycerine, and examining this with a high power (Zeiss $\frac{1}{18}$ in.), it was found that a variety of particles of considerable size, such as pollen grains, spores of *Penicillium*, starch grains, etc., had been arrested; and also that the specimen contained a large number of spherical and rod-shaped bodies, which were supposed to be bacteria. A few days later, upon examining specimens of yellow fever blood spread upon thin glass covers, similar bodies were discovered. Photo-micrographs were made, which showed these minute spherical and rod-like bodies interspersed among the blood-corpuscles; and distinguished physicians, who have since inspected these photographs, have supposed, before hearing an explanation of their real nature, that they were really bacterial organisms. This was my own opinion when I first saw them, but I noticed that they did not seem to be in exactly the same focal plane as the blood-corpuscles. I therefore resorted to the simple expedient of washing the blood from the cover-glass and remounting this over a circle of cement. Upon now examining it with the same power, I found that while the blood-corpuscles had disappeared, these *pseudo-bacteria* still remained,—showing that they were attached to or imbedded in the thin glass cover. I have since examined numerous glass covers that had been thoroughly cleaned by means of nitric acid, first, and distilled water or alcohol afterwards, and not infrequently I have found these same objects, which are only to be seen by the use of high powers.

But this is perhaps an unwarrantable digression, and I proceed to quote from the author mentioned:

> "These same elements were found in the vomited matter, having a white or greenish-yellow color, being especially abundant in large mycelial threads. In some cases there were ovoid cells, which appeared to be due to the alcoholic fermentation described by Pasteur. In these liquids, the spherical, yellow and elementary granules suffered the same changes as already noticed in the urine. The black vomit sediment appeared to be formed for the greater part of blackened mycelial threads, and other bodies of different forms and sizes, also black. There were also present yellow or greenish threads and elemental granules."

To this *fungus* of many forms and many colors the discoverer has given the name "*Peronospera lutea*."

The writer failed to find anything corresponding with this description in his examinations of blood, urine, and black vomit, while in Havana, but reports as follows:

"Organic fluids, such as urine, black vomit, and the fluid from the interior of unripe cocoanuts, exposed in the laboratory, very soon became filled with a variety of vegetable organisms, bacteria, torulæ, vibriones, and other fungi, such as are found under similar circumstances in all parts of the world. Most of these were well-known and common forms; some may have been peculiar to the latitude or even to localities infected with yellow fever, but to decide this question would require a more precise knowledge in regard to these low forms of vegetable life than was possessed by any member of the Commission, or, indeed, than is likely to be found even among those who have devoted the most attention to this branch of study, which is acknowledged by all to be yet in its infancy.

"Photo-micrographs were made of some of these forms, and it is suggested that photographic representations of all forms found in southern parts of the United States at a time when yellow fever does not prevail, should be made in advance of the next epidemic, so that any unusual form presenting itself then may receive the special attention of future investigators" (*l. c.*).

In Fig. 4, Plate II., and in Figs. 1 and 2, Plate III., of the present volume, photographs from nature are given of some of the organisms which were found most abundantly in yellow fever urine. It may be that one of these, or some one of the many organisms which Carmona has included in his description, is the veritable germ of yellow fever; but this is a mere hypothesis not supported by the slightest evidence. At the time of my visit to Havana I had not perfected my method of conducting culture experiments (see p. 178), and if I had been fully prepared for the work, could not have found the time to obtain pure cultures of each micro-organism encountered, and to make inoculation experiments for the purpose of determining whether any one of them had specific pathogenic properties. Pasteur was engaged for several years in his study of *pébrine*, the parasitic disease of silkworms upon which he may be said to have founded his scientific reputation. That the etiology of yellow fever was not worked out during the three months' stay of the Havana Commission in Cuba cannot therefore appear surprising to those who know the difficulties of such an undertaking; and if Dr. Carmona, or Dr. Somebody-else succeeds in carrying off the laurels due to a discoverer, it will be rather a matter of luck than of science, unless he attacks the problem by the painstaking and timetaking methods which have been perfected by Pasteur, Koch, and other pioneers in this line of investigation. Dr. Carmona says:

"If a portion of urine be allowed to evaporate spontaneously, and the residue be examined microscopically, the protoplasmic substance containing abundant spherical yellow granulations, mycelial tubes, and crystals of cholesterine and tyrosine, before mentioned, are seen. The free extremities of many of the mycelial threads were gradually dilated, somewhat resembling the extremity of the olfactory bulb." These dilated extremities Carmona calls *oögonos*, and they measure from 10 to 60 μ.

Yellow fever urine is an acid albuminous fluid, and a suitable culture-medium for a variety of bacterial organisms and microscopic fungi. At the extremity of the urethral canal, bacteria are always found in considerable numbers, and the urine of healthy persons, or of yellow fever patients, is necessarily contaminated with these when it is voided. Urine "allowed to evaporate spontaneously" is presumably exposed to the air and to inoculation with the numerous germs which it contains.

Dr. Carmona says: "The black vomit sediment appeared to be formed for the greater part of blackened mycelial threads, and other bodies of different forms and sizes, also black."

The uniform testimony of competent microscopists who have heretofore examined black vomit is, that the dark color is due to the presence of blood, altered by the acid secretions of the stomach, which escapes from the hyperæmic mucous membrane during the later stages of the disease, when passive hemorrhages are common. The writer has repeatedly verified this fact, and, while in Havana, made photo-micrographs, which show that the little dark-colored flocculi in the vomited material are made up of decolorized blood-corpuscles and of amorphous masses of dark material which is presumably hæmoglobin from these decolorized corpuscles, changed by the acid secretions of the stomach. A microscopic examination of black vomit or of the transparent acid fluid ejected at frequent intervals before hemorrhages occur, shows that it contains epithelium from the mouth and bacteria of various forms. This is not surprising when we remember that every drop of saliva swallowed is charged with a variety of these minute plants. To decide whether any one of these bears a causal relation to the disease, would require extended culture-experiments, and the administration of a pure culture to man himself, as a test of specific pathogenic power, unless satisfactory evidence can be obtained that some one of the lower animals is susceptible to the disease.

A more recent claim to the discovery of the yellow fever germ is that made by Dr. Freire of Brazil.

I quote again from the "Medical News" (July 7, 1883, p. 13):

> "Dr. Freire recognizes in the blood of yellow fever patients a cryptococcus to which he has given the specific title of *Xanthogenicus*. In the phases of its development it appears as minute points, or as large round cells with grayish or fringed margins, and bright transparent centres. Besides these there are occasionally seen transparent granulations, aggregated in a yellowish matrix. A gramme of blood charged with these organisms, from a yellow fever patient, was injected into the veins of a rabbit, which died in a quarter of an hour with tetanic convulsions. . . . At the autopsy visceral congestions were found, similar to those seen in persons dead of yellow fever, and the blood was found to contain the cryptococcus which was present in that which had been inoculated.
>
> "A gramme of the blood of this rabbit was injected

hypodermically into a guinea-pig, which died at the end of some hours. Its blood was found to contain an extraordinary quantity of the cryptococcus. A second guinea-pig was inoculated by hypodermic injection with the blood of the first one, and after some hours the animal appeared feverish and oppressed, with cold ears and paws, trembling, and blackish vomiting. It died in a short time, and its blood showed an infinity of the characteristic organisms.

"Dr. Freire considers that these experiments establish the parasitic nature of yellow fever, and that the parasite *C. Xanthogenicus*, is found in every undoubted case of the disease. He has also discovered and isolated the alkaloid from the black vomit, which he regards as a product or excretion of the microbes. He considers that the color of black vomit is not due to altered blood, but to the cryptococcus. He regards cemeteries as perennial foci of the disease. Some earth was taken from the grave of a man who had been buried a year before. A guinea-pig shut up in a confined space with this earth died in five days. Its blood was literally crammed with the cryptococcus in various stages of evolution; its urine was albuminous, and its brain and intestines yellow with the peculiar pigment of the microbe."

The writer is not prepared to estimate the value of the evidence here offered, inasmuch as we are not informed whether the yellow fever blood used in the first inoculation experiment was obtained *post mortem* or *ante mortem.* It would be interesting also to know whether the cryptococcus was obtained in blood drawn with proper precautions during the lifetime of the patient. While in

Havana, the writer paid very little attention to *post mortem* blood; but it was noticed that in blood drawn during the last hours of life the serum was tinted yellow, and the red corpuscles were paler than normal from a loss of hæmoglobin. Any albuminous granular material in *post mortem* blood — from disintegration of the corpuscles, etc. — would therefore be likely to be stained yellow by this pigment.

Hineman, a very competent German physician practising in Vera Cruz, has not been more successful than the writer in finding the *Peronospera lutea* of Carmona, or the *Cryptococcus Xanthogenicus* of Freire, in the blood of yellow fever patients, before death. He examined the blood of patients in the last stage of the disease, taking blood from the hand, thinning it with artificial serum, *and bringing it at once* under the microscope. He says: "In nine cases so examined not the slightest deviation from normal blood could be found. . . . No organisms were found."[1]

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[1] Arch. f. path. Anat. LXXVIII. p. 139.

PART SIXTH.

BACTERIA IN SURGICAL LESIONS.

The important part played by bacteria in surgical lesions can no longer be questioned. This is demonstrated (*a*) positively, by the ill-effects which result from the retention of discharges containing putrefactive bacteria upon the surface of open wounds, or in sinuses and cavities; and (*b*) negatively, by the favorable results of antiseptic treatment; and the fact that when the access of micro-organisms is prevented by the integrity of the cutis, very severe lesions, attended with an abundant exudation of bloody serum, are commonly recovered from without suppuration or any evil result from the resorption of this fluid and of inflammatory exudates. But this same material quickly attains poisonous properties in the presence of bacteria, and not only exercises a deleterious local effect, unfavorable to the repair of the injury, but its absorption now is attended with the most serious consequences.

These facts, which are so generally recognized that it is unnecessary to present evidence in their support, are in accord with the following propositions which have been established by experimental research and may be accepted as fundamental truths upon which to base our reasoning as regards the *rôle* of the bacteria in surgical lesions.

(*a*) The blood and tissues of healthy persons do not, under ordinary circumstances, contain bacterial organisms.

(*b*) Putrefactive decomposition of organic fluids is due to bacterial organisms.

(*c*) Albuminous fluids, — e. g., blood and pus, which have undergone putrefaction, contain a potent poison, or poisons, which, in comparatively small amount, may produce death in the lower animals.

We have here a sufficient foundation for the antiseptic treatment of wounds. But in addition to this there are strong reasons for believing that certain species of bacteria have also the power of invading the tissues, and producing local necrosis, when for any reason the vital resistance of these tissues is reduced, — e. g., from hemorrhage, from starvation, from crowd poisoning, *from septic poisoning*. Or the same result may perhaps occur when the vital resistance of the tissues is not below par, in consequence of the unusual vigor of the micro-organisms, developed as a result of unusually favorable conditions of environment. As, for example, when a healthy man, recently wounded, falls a victim to hospital gangrene as the result of infection in a crowded ward, in which this infectious disease was in the first instance developed *de novo.*

The purulent discharge from wounds not treated antiseptically always contains micro-organisms. These are mainly micrococci and short rods like those shown in Figs. 28 and 29, which are copied from Cheyne's recent work on "Antiseptic Surgery."

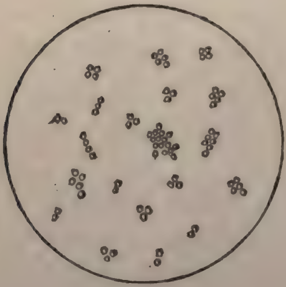

Fig. 28.

Micrococci from a wound treated aseptically, growing in infusion of cucumber. × 1450. (From Cheyne's "Antiseptic Surgery.")

The micrococci represented in Fig. 28 were obtained by cultivation in cucumber infusion, from a wound treated aseptically. The organisms represented in Fig. 29 are from a case of compound dislocation of the thumb not treated aseptically. The rod-bacteria in this figure are doubtless septic bacteria, properly so called, which give rise to the putrefactive decomposition of albuminous fluids. The observations of Cheyne show that these may be excluded from the secretions of wounds by antiseptic treatment, and that, in this case, the pus discharged from such wounds presents no evidence of putrefaction, although, in certain cases, micrococci are found in this pus formed beneath antiseptic dressings. This is explained by the greater resisting power of micrococci to antiseptic agents. Cheyne says:

"Micrococci prefer acid fluids; most bacteria prefer alkaline or neutral fluids.

"Micrococci grow, readily, in fluids containing proportions of carbolic acid in which bacteria only grow with difficulty" (*l. c.*, p. 244).

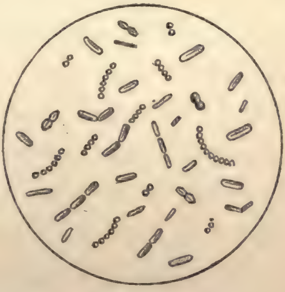

Fig. 29.

Specimen of discharge taken from a case of compound dislocation of the thumb not treated aseptically. × 1450. (From Cheyne's "Antiseptic Surgery.")

The experiments of the writer have not shown any difference as regards the action of carbolic acid in preventing the development of these different organisms in culture-fluids; but in the case of boric acid and of sodium biborate a very marked difference was observed, the micrococcus of pus developing freely in the presence of 0.25 per cent of boric acid, while *B. termo* failed to develop in the presence of one-half this amount. It is probable that free access of oxygen in the culture-experiments, and its exclusion, to some extent at least, from the surface of wounds treated antiseptically by Lister's method, is an advantage in favor of the micrococcus in the latter case; for we know that this may multiply freely in the absence of oxygen in the pus of a closed abscess.

While there is no question as to the injurious effects of putrefactive bacteria in the discharges from wounds when these are retained upon an absorbent surface, or in a sinus or pus-cavity, the *rôle* of the micrococcus of pus has not been so well established. According to one view, inflammation results in the formation of pus only when this micrococcus is present, and because of its presence. On the other hand, it has been claimed that it is simply present because it finds in pus a suitable culture-medium, and that its presence in this fluid is without significance. Cheyne is inclined to look upon this micrococcus as comparatively harmless; and without doubt it may be present in the pus secreted by wounds which are healing in a most satisfactory manner. Cheyne says:

"It is certain that they do not cause putrefaction, but they always cause a sort of sour, sweaty smell in fluids, — a smell which can be recognized in whatever fluid they grow: in other words, they are associated with a peculiar fermentation. Now, the products of this fermentation are but little irritating. They have no acrid taste, nor do they feel pungent when applied to a cut surface. Hence, probably, it is that we find wounds in which these organisms exist, even in large numbers, appear often unaffected by their presence.

"Nevertheless, they can hardly, under any circumstances, be indifferent, and I think I have observed that in some cases, after they have got in, the wounds do not behave quite as typically as usual; *i. e.*, there may be a trace of suppuration, or a sinus takes longer to heal than one had any reason to expect."

To test the possible local pathogenic action of the micrococcus of pus the writer made the following experiment:

"*Exp. No.* 12. — August 4. — An incised wound was made with scissors, removing a fragment of skin upon each thigh of a half-grown rabbit. The wound upon the right thigh was moistened with a culture-fluid (twentieth culture) containing the micrococcus from gonorrhœal pus. The wounds were then dressed with dry tow, and a bandage applied. Both healed kindly without any undue inflammation, and no difference was observed between the two."

This single experiment counts for but little; and the criticism may be made that this micrococcus was obtained from *gonorrhœal* pus, and is perhaps specifically distinct from the micrococcus of ordinary pus, although it appears to be morphologically identical with it. All this is admitted, and the experiment is introduced mainly to call attention to a method, which, carefully applied, should enable us to solve the question as to the pathogenic *rôle* of this micrococcus. The writer had mapped out for himself a series of experiments in this direction and many others relating to etiological questions, but circumstances have not been favorable for the prosecution of experimental work, and he finds himself, somewhat reluctantly, engaged in a review of the field, when it would be far more to his taste to interrogate nature by the experimental method, and thus to aid directly in the solution of these interesting problems.

One of these problems, with our present light, is very puzzling. It has been demonstrated by numerous observers that this micrococcus of pus is uniformly found in pus obtained from an acute abscess, when the integument covering it is still intact, even when it is deeply situated in the tissues; and yet the observations of Pasteur, Koch, Cheyne, and many others, are in accord as to the absence of all micro-organisms from the blood of healthy persons. Whether, then, we suppose this micrococcus to be the cause or the result of the formation of these abscesses, we are met by the question, How did it get there in the first instance? In certain cases such abscesses may be traced to an injury in which a slender, sharp-pointed instrument — e. g., a needle or a thorn — has penetrated deeply into the tissues; and in this case we may suppose that micrococci have been introduced in this way. Or possibly the point of inoculation may have been far removed from the situation where the abscess is developed, and the organisms may have made their way in the blood-current or through the lymphatics to this point, where, for some mechanical reason, they have been arrested. But this is speculation, and we must leave the question unsettled, and content ourselves for the present with a summary statement of the observed facts relating to the presence of this micrococcus in collections of pus not exposed to the air.

In 1875, Bergeron, in a communication to the French Academy of Sciences, reported, as the result of numerous observations made for the purpose of ascertaining if the pus of abscesses contains bacteria, as follows:

"1. Vibrios are found in the pus of abscesses, without any contact with the external air, and without, usually, any indication that the organism is seriously affected by their presence. 2. We cannot admit that in these cases the vibrios have penetrated into the interior of the abscess through the lymphatic system, or through the circulating system, both being intact. The pus of warm abscesses in adults often contains vibrios; if they occur in the case of infants the fact has not been observed. 3. The pus of cold abscesses in the adult, as in the infant, never contains them." (Magnin.)

The vibrios of Bergeron are doubtless identical with the micrococcus described by later observers, which often occurs in chains. The observations of Billroth, Cheyne, and Ogston, are in accord with those of Bergeron as to the presence of micrococci in acute abscesses, and their absence from chronic abscesses. Cheyne has shown, however, that when these organisms are proved to be present in pus from an abscess, by microscopical examination, this pus often fails to fertilize a culture-fluid, thus proving that the micrococci are no longer living. He says:

> "Of acute abscesses, I had up to May, 1879, inoculated from thirty-two cases. In twenty-five of these no growth of organisms occurred, while from six micrococci were obtained. In no case did I get bacteria" (*l. c.*, p. 253).

Ogston examined the pus from eighty-two abscesses, all of which had been "hitherto unopened." The pus was taken from them by means of a needle or a knife while still flowing from the incision, spread out in a thin film upon a slide, immediately dried, and stained with an aniline dye. Of the abscesses examined, thirteen were —

> "Chronic typical cold abscesses, whose duration could be measured by months, proceeding from chronic carious disease of bone, scrofulous lymphatic glands, and such like. In none of them were any organisms found.
>
> "Four were somewhat chronic abscesses, whose duration could be measured by weeks; and which had followed diseases more or less allied to, or complicated with, forms of blood-poisoning and hectic, such as tonsilitis, phthisis, scarlatina, erysipelas, typhoid fever, and diphtheria. All of these contained micrococci, and were evidently the same as the next form.
>
> "Lastly, sixty-five were acute abscesses, whose duration could be measured by days, from all parts of the body. Every one of these contained micrococci."

The cocci were found (*a*) in chains, usually of five or six elements, but often much longer — in one case three hundred and twenty-one cocci were counted in a single chain; (*b*) in groups, "like the roe of a fish" — zooglœa masses; (*c*) in groups of three or four, "many of which were clearly, from the equal size and relative positions of the cocci, formed by a direct division into fours, or even, though more rarely, into threes;" (*d*) in some cases unusually large oval cocci were found, chiefly in pairs. "For the most part these varieties existed in separate abscesses, but it frequently occurred that an abscess contained both chains and groups. Out of sixty-four abscesses where this point was specially noted, seventeen contained chains only, thirty-one groups only, and sixteen both forms, or only pairs."

Ogston was unable to discover any difference in the character of the abscesses which contained these different forms, and could not decide definitely whether they represented different species or only varieties of the same species.

To ascertain whether these micrococci possessed pathogenic properties, Ogston injected pus containing them into guinea-pigs and mice; and, for comparison, pus from cold abscesses, which contained no micro-organisms, into other animals of the same species.

The invariable result of twenty experiments, in which pus from the last-mentioned source was used, was that no illness or abscess ensued.

"But a very different effect was produced when similar injections were made with pus containing micrococci. In every instance, with the qualifications to be presently made, well-marked disease was set up. Quantities, varying from one to three minims, produced, in the animals already mentioned, symptoms of blood-poisoning, lasting from two to five days. . . . These symptoms became less marked towards the end of the first week or five days. If the animal was killed during this stage, the blood in its right heart was found to contain micrococci; single, in pairs, and in short chains of six or fewer, swimming in the serum between the cells. Around the site of injection was found a patch of red infiltration, varying in size, and having in its centre more pus than corresponded with the quantity that had been injected. The pus contained myriads of micrococci of the same nature as those injected, but more numerous. . . . The cocci were living and growing, and a drop of the matter injected into another animal produced the same results in it, and it on another animal, and so on. No increased virulence was observable in the transferrence through a series of animals. The red infiltration around the abscess showed the micrococci invading the neighboring tissues, penetrating between their cells, and in colonies or chains, gradually decreasing in size, pushing their way for a considerable distance into the structures in the vicinity. . . . After five to seven days had elapsed, and in some cases even earlier. the animals exhibited a change. They became more active again, threw off their lethargy, and seemed well; but at the spot where the injection had been made, there was found a fluctuating tumor, gradually increasing in size, and presenting all the signs of being an ordinary abscess. When they were killed during this second stage, micrococci were more rarely found in the heart-

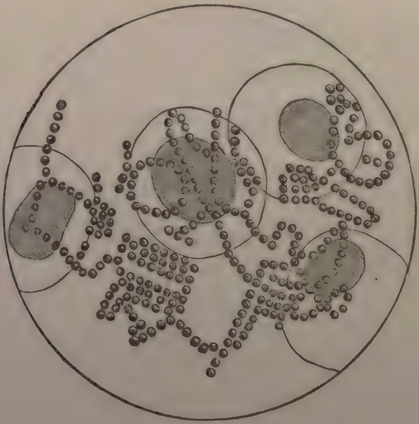

Fig. 30.

Group of chain micrococci in pus. × 1600. (Ogston.)

blood, and the infiltration of the organisms into the tissues around the abscess no longer existed, having been replaced by a firm, thick wall of granulation tissue, in which micrococci could seldom be detected, and which seemed to act as a barrier, preventing or diminishing their migration into the blood and surrounding structures. . . .

"On the presumption that carbolic acid would destroy the power of the micrococci, a series of injections were instituted with pus mixed with equal parts of a five per cent watery solution of that substance. These were employed on separate animals, as well as on a different part of an animal injected with unmixed pus from an acute abscess; and in every case, the pus so disinfected, though injected in larger quantity, produced no reaction whatever, but disappeared in the rapid and complete way described under the experiments with that from cold abscesses.

"I next endeavored to ascertain the temperature capable of destroying the power of micrococci. Although, in this direction, the experiments were not so numerous as is desirable, it may be stated that pus heated to 130° Fahr., or higher, hitherto always failed to excite suppuration."

This is strong evidence in favor of the view that the formation of acute abscesses is due to the presence of this micrococcus. The writer has shown that its thermal death-point is 140° Fahr., the time of exposure being ten minutes. Ogston does not state the time of exposure to a temperature of 130° Fahr., but it may well be that a somewhat longer exposure than ten minutes at this temperature would also be fatal to the micrococcus.

Ogston made also a large number of culture-experiments.

"As might have been anticipated, cultivations of pus of cold abscesses (five cases) yielded uniformly negative results.

"Cultivations of pus of acute abscesses gave at first the most inexplicable and contradictory results. This was ascribed to the fact that the micrococcus in question is *anaérobie*, and cannot grow in the presence of oxygen. The plan was therefore tried of growing them in eggs. Newly-laid eggs were washed in five per cent carbolic water; and, under spray, a minute aperture was pierced in the larger end. One minim of pus from an acute abscess, collected under the strictest antiseptic precautions, was injected by a long-pointed pure syringe into the albumen at the opposite end of the egg. A piece of protective was laid over the aperture. The egg was enveloped in a Lister's dressing, and kept for ten days in the incubator at 98° Fahr. At the end of that time it was opened, and my expectations were fulfilled. The egg was sweet and fresh; its contents were unaltered, save the yolk was somewhat broken up, and more or less mixed with the albumen; but the albumen, and sometimes the yolk also, were filled with enormous chains or masses (according to the sort of coccus used) of micrococci, growing quite as luxuriantly as I had ever observed them when experimenting on animals. A drop of the albumen injected into an animal's back now produced typical abscess, with all the symptoms already mentioned; and the animal, on being killed, showed the micrococci in the blood and invading the tissues, exactly as had been already obtained by the employment of the pus of acute abscesses."

This is an extremely interesting experiment, but Ogston is evidently mistaken in ascribing the contradictory results at first obtained to the fact that the micrococcus in question is *anaérobie;* for while this is true, and it can doubtless grow in the absence of oxygen, the writer has found no difficulty in cultivating it through successive generations, in the culture-flasks described on page 177, in *bouillon* made from the flesh of a rabbit or of a chicken, and in the presence of atmospheric air, with which these flasks are two-thirds filled when prepared in the manner indicated. Thus, in my experiments upon the germicide power of various therapeutic agents, a pure-culture of this micrococcus was maintained through many successive generations, culture No. 1 having been inoculated with a drop of pus from a whitlow, obtained at the instant of its escape from a deep incision. The true explanation of the contradictory results obtained by Ogston is doubtless that given by Cheyne, viz.: that when no development occurred in culture-solutions inoculated with the pus of acute abscesses, it was because the micrococci were already dead. Wernich has shown that during the multiplication of various bacterial organisms in a limited amount of nutritive pabulum, chemical products are evolved fatal to the vitality of these organisms.

In conclusion, the writer would suggest that those who desire to make themselves familiar with the organisms to which a pathogenic *rôle* has been ascribed, and with the technique relating to their recognition, cultivation, etc., will do well to commence with this micrococcus of pus, a pure culture of which may be easily obtained in the manner heretofore indicated.

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